GEOGRAPHIC SONG VARIATION AND DAWN SINGING BEHAVIOR OF THE CERULEAN WARBLER (*SETOPHAGA CERULEA*)

A THESIS

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CHAPTER 1: Geographic uniformity in song structure within the Cerulean Warbler (*Setophaga cerulea*) core breeding range

1. ABSTRACT

The Cerulean Warbler (*Setophaga cerulea*) is one of the fastest declining North American wood-warblers (family Parulidae); however, little is known about how consistent or variable its songs are across its breeding range. Geographic song variation may be informative about patterns of population connectivity and dispersal, and since song functions in courtship and species recognition, it has the potential to promote speciation by acting as a pre-mating isolating mechanism. We examined geographic song variation in the Cerulean Warbler by measuring 26 acoustic and temporal variables from the songs of 233 individual male warblers from five Bird Conservation Regions (BCRs) spanning the core breeding range. All measurements were made using Raven Pro 1.5. We used audio recordings from public collections, colleagues, and from personal fieldwork in 2017. We assessed geographic variation in the 'wholesongs' and in each of the three distinct sections of each song through acoustic song variables relating to measures of frequency, duration, and song composition. We also examined if 'wholesongs' and song sections showed geographic structure among BCRs. Song structure was similar in several characteristics, including composition, duration, frequency, and bandwidth, both within a two BCR "core" and within an extended five BCR area. We failed to find evidence for geographically structured singing (i.e., dialects). Our study is the first to assess patterns of geographic song variation across the core breeding range of the Cerulean Warbler. Additionally, the lack of geographically structured singing supports the existence of gene flow between breeding populations, which may have significant implications for the conservation of the declining Cerulean Warbler.

Keywords: Cerulean Warbler, *Setophaga cerulea*, geographic variation, birdsong

2. INTRODUCTION

Geographic variation in the songs of oscine passerine birds, those species that learn their songs from neighboring male tutors, can provide insights into the evolutionary history of a species. Song variation may arise through a variety of mechanisms: by means of stochastic processes (i.e., drift or "cultural transmission"), when song-copying errors by young males lead to an emergence of new song variants in a population (Podos and Warren 2007), or through selection on bill dimensions or body size that influences the production of song (Grant and Grant 1996). Variation in song can also be a direct adaptation to differences in habitat structure ("acoustic adaptation hypothesis") whereby the potential of a given habitat to degrade and attenuate vocal signals can lead to the selection for songs that match the transmission characteristics of that habitat (Hansen 1979). Song variation can also arise due to influences from the human sound environment (e.g., anthropogenic noise; Slabbekoorn and Ripmeester 2007) or by various sources of natural or environmental sounds (e.g., insects; Kirschel et al. 2009). Because one of the primary functions of birdsong is in mate attraction and species recognition (Kroodsma and Byers 1991), differences in song may act as a pre-zygotic isolating mechanism, separating individuals and resulting in genetic differentiation. Populations that diverge in song may no longer recognize each other as conspecific, leading to assortative mating and reproductive isolation, and, potentially, speciation (Irwin et al. 2001).

There are several common patterns of geographic song variation in passerine birds, whose songs can be learned or inherited. Song conformity across the geographic breeding range occurs in the suboscines, those species with genetically determined songs, and also rarely in

oscines (e.g., Prothonotary Warbler *Protonotaria citrea*; Bryan et al. 1987). Another pattern is extensive between-individual variation with no population geographic structure (e.g., MacGillivray's Warbler *Geothlypis tolmiei*; Pitocchelli 1990). Further, songs may vary gradually across the breeding range (i.e., clinal variation), such as in the White-throated Sparrow (*Zonotrichia albicollis*), whose songs differ in the number of terminal notes across its Canadian breeding range (Ramsay and Otter 2015). Finally, 'dialects,' or song vocal neighborhoods, occur when song populations occupy discrete geographic regions. For example, some White-crowned Sparrow (*Zonotrichia leucophrys*) populations share the same song type but sing songs that differ from those of neighboring groups (microgeographic variation; Marler and Tamura 1962). 'Dialects' may also occur on more extensive, macrogeographic scales, such as in the Mourning Warbler (*Geothlypis philadelphia*), which is separated into four groups based on patterns of similar singing (Pitocchelli 2011).

Relatively little work has been conducted on geographic song variation in the Cerulean Warbler (*Setophaga cerulea*), an oscine passerine that breeds in deciduous hardwood forests in the eastern United States and southeastern Canada (Buehler et al. 2013). Cerulean Warbler populations have severely declined since the 1960s (Sauer et al. 2014), and many populations exist in increasingly isolated patches (Veit et al. 2005). The only previous study on geographic song variation in the species, by Woodward (1997), found minimal variation between individuals in Ontario and Illinois, though this study did not include songs from across the species' entire geographic breeding range. Knowledge about patterns of geographic song variation could be informative about the existence of population structure across the Cerulean Warbler breeding range and could have implications for population connectivity, dispersal and gene flow, conservation, and potentially for the species' management. The objective of our study was to

describe, for the first time, geographic variation in Cerulean Warbler song across the entire core of the species' breeding range.

3. METHODS

3.1 Male Song Structure

A typical male song consists of three distinct sections, each higher in frequency than the previous. The first and second sections are a series of complex two-element phrases, the first section approximately half the duration of the entire song and the second section much shorter, while the final section is a loose “buzz” or “trill”: a series of steady broad-frequency notes repeated rapidly over a short duration (Figure 1). Song sections may be added or dropped, such that a male’s song may have 2-4 sections. Males have 2-3 song types that are divided and used in different contexts for Type I and Type II singing, which are thought to target females and rival males, respectively (Woodward 1997).

3.2 Audio Recordings

We measured the songs of 233 individual male Cerulean Warblers from across the species’ breeding range. Each selected recording corresponded to a current or historically known breeding location within each region's nesting period (i.e., outside of migration). We grouped recordings by Bird Conservation Regions (BCRs), which are ecologically distinct regions with similar bird communities and habitats, and we analyzed geographic song variation with individuals grouped in two ways: in a two BCR "core" and an extended five BCR area. Sample sizes varied widely among BCRs (Figure 2). Recordings were obtained from the Cornell Lab of Ornithology's Macaulay Library (macaulaylibrary.org; n = 51) and eBird (eBird.org; n = 40),

Michigan State University's Avian Vocalization Center (avocet.zoology.msu.edu; n = 1), xeno-canto (www.xeno-canto.org; n = 24), the Ohio State University Borror Laboratory of Bioacoustics (blb.osu.edu; n = 55), as well as from colleagues (n = 5). We also included personal audio recordings obtained with a Marantz PMD661 MKIII solid-state digital recorder (48 kHz sampling rate, 24-bit) paired with a Telinga Pro Universal parabola and Sennheiser K6/ME62 omnidirectional microphone (n = 57). Recordings were made between 1951 and 2017. The majority of files were recorded in WAV format (n = 202), with the rest being MP3 (n = 31). Most recordings had a 48 kHz sampling rate; those that did not were transformed to 48 kHz for uniformity across the data set.

To measure acoustic variables, we selected recordings with a high signal to noise ratio (visualized from spectrograms) and minimal competing background noise. We measured acoustic variables in Raven Pro 1.5 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA) with a Hann window size of 420 DFT. Males have 2-3 song types representing Type I and Type II singing behaviors, but for this analysis, we pooled all recordings for two reasons: 1) one male's Type I song can be another male's Type II song and 2) Cerulean Warbler songs are not "form-encoded" and thus, not easily identified to type (Woodward 1997). We selected songs using a random number generator, and we attempted to measure three songs per male. However, due to variability in the quality of each recording, the number of songs measured per male ranged from 1-3. To avoid pseudoreplication, we treated the averaged values of the songs of each male as an individual unit of analysis, such that each male was considered a sample size of one.

We conducted two parallel analyses corresponding to variables concerning the ‘wholesong' (the entire song) and to each of the three distinct song sections (sections ‘A,' ‘B,' and

‘C'). For the ‘wholesong' analysis, we measured six acoustic variables associated with frequency and duration: the maximum and minimum frequencies as determined by the peak frequency contour (kHz), the duration and the 90th percentile duration of the song (the latter being more robust to measurement error; sec), the 90th percentile of the bandwidth of the song (the difference between the highest maximum and lowest minimum frequencies; kHz), and center frequency (the frequency at which 50% of the song energy is above and below; kHz). From spectrograms, we also visually assessed two additional variables corresponding to counts of the number of section types and the total number of sections of each song, for a total of eight acoustic variables for the ‘wholesong' analysis. For the analysis of the individual song sections, we measured the same first six acoustic variables from the ‘wholesongs' in each of the three song sections (leaving out number of section types and total number of sections), for a total of 18 variables for the song sections (six variables for each of the three song sections) and 26 variables overall. All variables are standard in acoustic studies of birdsong, and many of these same variables were used in the few previous studies of Cerulean Warbler song.

We grouped individuals by BCRs rather than by state and province boundaries, which are biologically arbitrary. We assessed geographic song variation within two separate groupings of BCRs: a two BCR area, Central Hardwoods and Appalachian Mountains, that represents the "core" of the species’ breeding range and that likely corresponds to the majority of breeding individuals, and an extended five BCR area, also including Eastern Tallgrass Prairie, Lower Great Lakes, and Prairie Hardwood Transition, that is broader in geographic extent and that covers most of the species' breeding range (Figure 2).

3.3 Statistical Analysis

All statistical analyses were carried out in Program R (R Development Core Team 2016) using the base R package unless otherwise noted. Alpha was set at 0.05. We tested the 26 acoustic variables of the ‘wholesong' (eight variables) and song sections (three sections, each with six variables) data sets for normality using the mvnormtest package in R (Jarek 2012). Variables not normally distributed were log or exponentially transformed to achieve normality. We conducted Bartlett's test for homoscedasticity and found that all variables met an assumption of equal variance. We scaled all variables (z-scores with each variable centered on 0 and scaled to unit standard deviation) before conducting further analyses.

We performed a principal component analysis (PCA) using the base R package on each of the parallel sets of eight ‘wholesong' and each of the six song section variables to reduce acoustic variables into smaller sets of principal components (PCs) and also to examine the individual and combined effects of multiple variables on song structure variation. Principal components with eigenvalues greater than 1.00 were retained for use in subsequent analyses. Differences in song structure between regions were assessed via separate MANOVAs for the ‘wholesong' and each song section using the retained PCs. If the resulting p-value from the MANOVA was significant, we followed up with ANOVAs and Bonferroni-corrected post-hoc comparisons of the between-group effects of each of the individual principal component response variables. To test whether song structure could be used to accurately predict recording (and presumed breeding) location (BCR), we conducted a series of linear discriminant analyses (LDA) using the same principal components with the MASS package in R (Venables and Ripley 2002). The grouping variable was the BCR where each individual was recorded.

4. RESULTS

4.1 Song Structure

Most songs were composed of three distinct sections ('A,' 'B,' and 'C;' mean: 2.72, range 2-4), and each section was typically higher in frequency than the preceding one (Figure 1). Section 'A' averaged 0.17 sec (range 0.08-0.43 sec), section 'B' was typically shorter in duration (mean: 0.08 sec; range 0.04-0.14 sec) and was shifted slightly higher in frequency, while section 'C' was a "trill": a rapid repetition of a single broad-frequency note, and averaged 0.28 sec (range 0.06-0.51 sec). Average song duration across all individuals was 1.38 sec (range 0.85-1.95 sec). Most songs were between 3.5 and 7.0 kHz in frequency (range 2.8-8.0 kHz).

4.2 Principal Component Analysis

For the 'wholesong,' the first three principal components were retained and explained about 71% of the variance (Table 1). The first principal component (PC1; eigenvalue 2.40) described a gradient from duration (sec; positive values) to minimum frequency (kHz; negative values), the second (PC2; eigenvalue 1.93) separated maximum frequency (kHz; positive values) from the number of section types (negative values), while the third (PC3; eigenvalue 1.37) described a gradient from minimum frequency (kHz; positive values) to the 90^{th} percentile bandwidth (kHz; negative values).

Two principal components were retained for section 'A,' two for section 'B,' and three for section 'C.' The two principal components for section 'A' explained about 84% of the variance (Table 2). The first principal component for section 'A' (PC1; eigenvalue 3.97) described a gradient from the 90^{th} percentile duration (sec; positive values) to maximum frequency (kHz; negative values) while the second principal component (PC2; eigenvalue 1.09) separated duration (sec; positive values) from minimum frequency (kHz; negative values). The two principal components for section 'B' explained about 85% of the variance (Table 3). The

first principal component (PC1; eigenvalue 3.78) described a gradient from minimum frequency (kHz; positive values) to duration (sec; negative values) while the second principal component (PC2; eigenvalue 1.30) separated the 90th percentile bandwidth (kHz; positive values) from minimum frequency (kHz; negative values). Finally, the three principal components for section 'C' explained about 89% of the variance (Table 4). The first principal component (PC1; eigenvalue 2.58) described a gradient from center frequency (kHz; positive values) to duration (sec; negative values), the second principal component (PC2; eigenvalue 1.50) represented maximum frequency (kHz; positive values) to minimum frequency (kHz; negative values), while the third principal component (PC3; eigenvalue 1.27) separated the 90th percentile bandwidth (kHz; positive values) from minimum frequency (kHz; negative values).

4.3 Power Analysis

We conducted a power analysis to determine the ability of our study to discriminate differences in song structure between geographic regions. For a one-way ANOVA comparing five groups (BCRs) with the following parameters: k = 5, f = 0.25, n = 15 (our minimum sample size for the five BCR comparison), significance level = 0.05, the power to discriminate differences between groups was 0.35. Similarly, for a one-way ANOVA comparing two groups (BCRs) with the following parameters: k = 2, f = 0.25, n = 83 (our minimum sample size for the two BCR comparison), significance level = 0.05, the power to discriminate differences between groups was 0.89.

4.4 'Wholesongs'

MANOVA

Overall, acoustic variables pertaining to the 'wholesong' differed between individuals breeding in the five BCRs (Wilk's $\lambda = 0.90$, $p = 0.02$), but none of the individual principal components revealed differences between groups (Figure 3). For the two BCR analysis, acoustic variables pertaining to the 'wholesong' did not differ (Wilk's $\lambda = 0.98$, $p = 0.34$; Figure 3).

Linear Discriminant Analysis

Linear discriminant analysis performed poorly at classifying 'wholesongs' to the correct BCR. For the five BCR analysis, accuracy ranged from 0-62.1% for individual BCRs, while overall accuracy was 37.3% (Table 5). For the two BCR analysis, accuracy was 38-62% for the individual regions, while overall accuracy was about 50% (Table 6).

4.5 Song Sections

MANOVA

There were no differences in acoustic variables pertaining to sections 'A', 'B', or 'C' for either the five BCR analysis (section 'A' Wilk's $\lambda = 0.98$, $p = 0.84$; section 'B' Wilk's $\lambda = 0.96$, $p = 0.40$, section 'C' Wilk's $\lambda = 0.93$, $p = 0.28$; Figures 4, 5, and 6, respectively) or the two BCR analysis (section 'A' Wilk's $\lambda = 0.99$, $p = 0.54$; section 'B' Wilk's $\lambda = 0.98$, $p = 0.22$, section 'C' Wilk's $\lambda = 1.00$, $p = 0.97$; Figures 4, 5, and 6, respectively).

Linear Discriminant Analysis

Linear discriminant analysis performed poorly at classifying song sections to the correct BCR. For the five BCR analysis, accuracy ranged from 0-60.5% for section 'A' (overall 36.9%), 0-68.8% for section 'B' (overall 39.4%), and 0-86.7% (Appalachian Mountains = 86.7%) for section 'C' (overall 32.8%; Table 5). For the two BCR analysis, accuracy was approximately 36-87% for the song sections, with an overall accuracy of 45-50% (Table 6).

5. DISCUSSION

We found that Cerulean Warbler song structure within the species' core breeding range (two BCR area: Appalachian Mountains and Central Hardwoods), where we had high power to discriminate differences, was highly uniform in all of the acoustic variables we measured. Songs were remarkably constrained in their acoustic features: all songs were composed of 2-4 section types and had similar durations and frequency bandwidths. The only previous research on geographic song variation in the Cerulean Warbler found that variation was minimal, though this analysis only included about 60 individuals from Illinois and Ontario (Woodward 1997). Our study was more robust in extent, though future research should focus on including recordings of individuals from more peripheral breeding locations. This level of uniformity across a large geographic area (covering two BCRs and multiple states) is fairly unusual in an oscine songbird, whose songs tend to vary due to drift or as a result of a suite of potentially interacting selective forces. We were not able to differentiate and analyze separately Type I and Type II songs, and thus it is possible that a different pattern would have emerged had we been able to do this. However, we believe pooling all recordings is appropriate, regardless of singing behavior, since one male's Type I song can be another male's Type II song (Woodward 1997). We did find differences between groups in variables pertaining to the 'wholesong' in the five BCR analysis; however, our sample sizes were limited by the number of available archived recordings, and we had less power to say if this effect was driven by small sample sizes or by actual among-group differences. Thus, recordists interested in Cerulean Warbler geographic song variation should focus on obtaining recordings away from the species' core breeding range.

When assessing the potential for song structure differences, it is prudent to compare patterns of acoustic structure with known patterns of genetic structure. In the Cerulean Warbler,

there is no evidence for population genetic structure, as assessed in microsatellite loci and a portion of the mitochondrial region of five breeding populations (Veit et al. 2005). Similarly, there are no recognized subspecies and there has been no plumage variation previously noted (Buehler et al. 2013). Geographic uniformity in song structure, particularly the robust result in the two BCR "core" area, could be considered congruent with our knowledge about a lack of population genetic structure in this species. There may be movement of adult males between breeding locations such that song mixing occurs within BCR regions. Geographic song uniformity may suggest that dispersal and gene flow is occurring between populations in the two BCR "core," since individual males presumably learn their songs from adult male tutors shortly after hatching and are potentially not able to alter their songs post-crystallization.

Future recordists should focus on obtaining audio recordings from the more peripheral breeding locations outside of the core breeding area (i.e., those outside of either the five or two BCR groupings that we analyzed). For instance, Cerulean Warblers also breed in the Atlantic Northern Forest, Boreal Hardwood Transition, New England, and Piedmont BCRs, but low sample sizes of available recordings precluded our ability to include them in this analysis. While we feel confident that songs do not differ in the acoustic variables that we measured in the two BCR region "core," we did find between-group differences in the five BCR analysis. It is possible, therefore, that songs in these more peripheral breeding areas differ from song in the core breeding area. Additionally, future researchers may consider assessing patterns of song structure on a population basis, rather than grouping individuals by BCRs. Breeding populations of Cerulean Warblers are highly fragmented and isolated in many areas, and treating populations as separate units may allow detection of between-population differences.

Studies of geographic song variation are sometimes accompanied by song playback experiments to determine the reactions of individuals to song from allopatric regions. Since song can act as a pre-mating isolating mechanism, differences in song can promote divergence between populations. Songs thus has the ability to play a major role in speciation. However, geographic song variation is often thought to be more informative in suboscines, those species with genetically determined vocalizations. Since oscines learn their songs, differences in song may not represent actual genetic differences. Future research should focus on elucidating patterns of song discrimination among different populations of Cerulean Warblers as such knowledge could have important consequences for the conservation and management of this declining species.

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CHAPTER 1 FIGURES

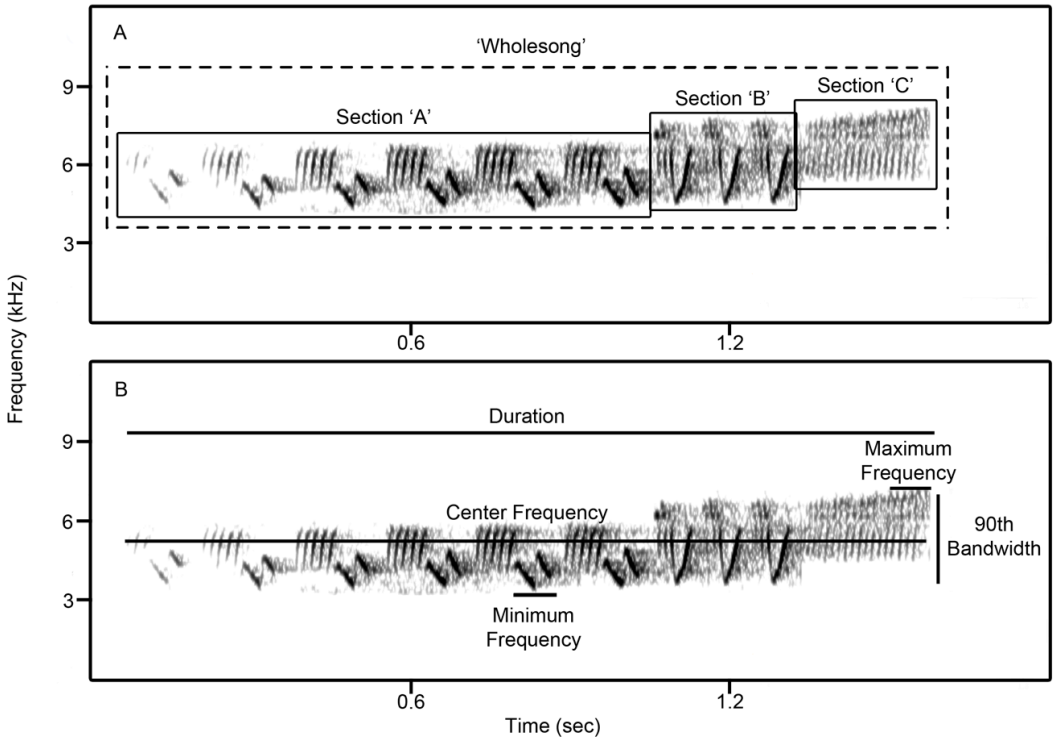

Figure 1. Spectrograms of a typical song of a male Cerulean Warbler showing the three song sections (solid boxes) and the 'wholesong' (dashed box; A), as well as the acoustic variables measured (B). Variables include the maximum and minimum frequencies (kHz), the duration and the 90th percentile duration of the song (the latter is more robust to measurement error; sec), the 90th percentile of the bandwidth of the song (the difference between the highest maximum and lowest minimum frequencies;), and center frequency (the frequency at which 50% of the song energy is above and below; kHz). Not shown is the 90th percentile duration.

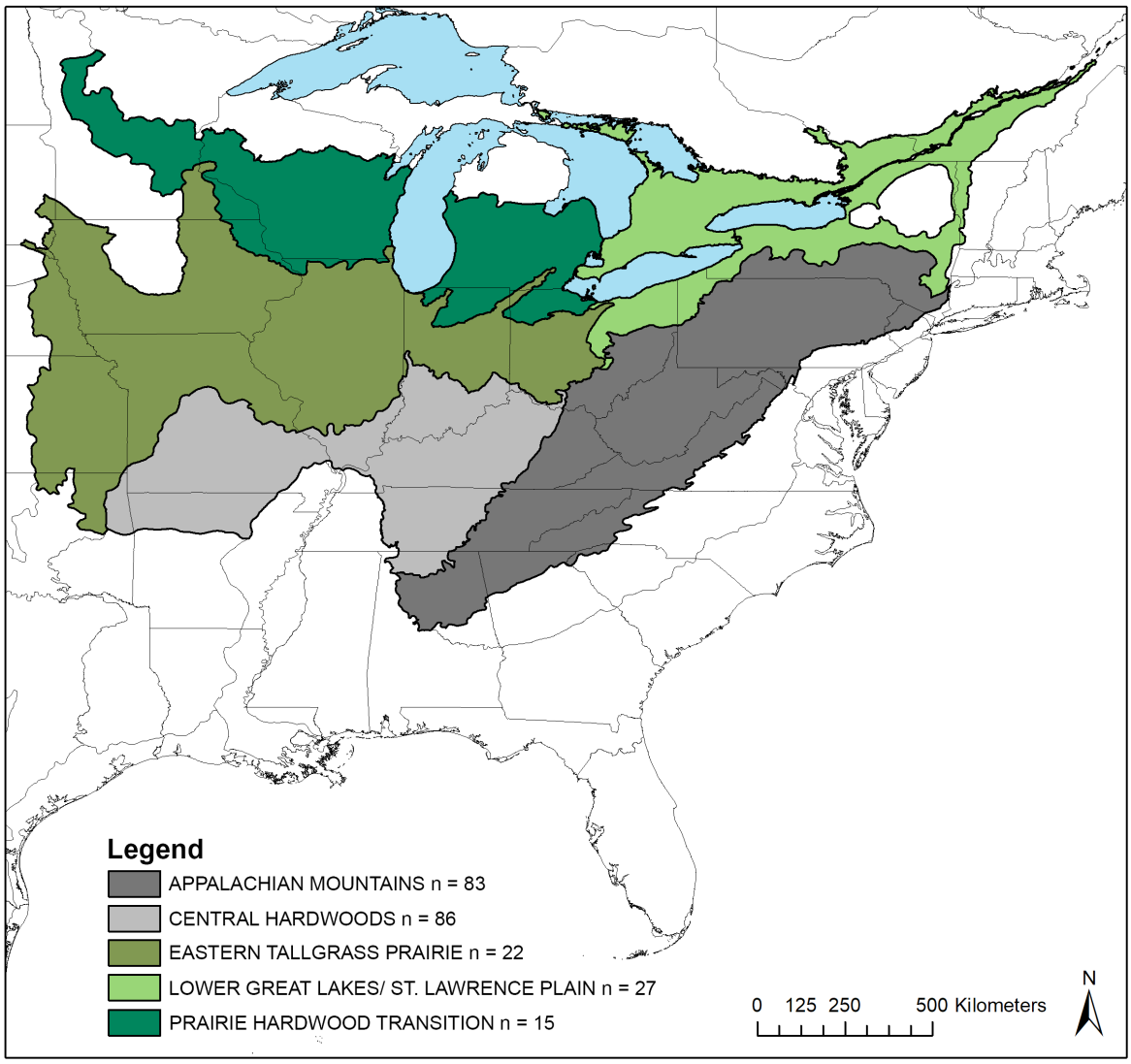

Figure 2. Map of the five Bird Conservation Regions (BCRs) used in our analysis of geographic variation in song of the Cerulean Warbler. BCRs shown in light and dark gray tones represent the "core" breeding area and pertain to the Appalachian Mountains and Central Hardwoods. An extended five BCR area is represented by the addition of the BCRs with light and dark green tones: Eastern Tallgrass Prairie, Lower Great Lakes, and Prairie Hardwoods Transition. Sample sizes indicate the number of measured audio recordings for each BCR.

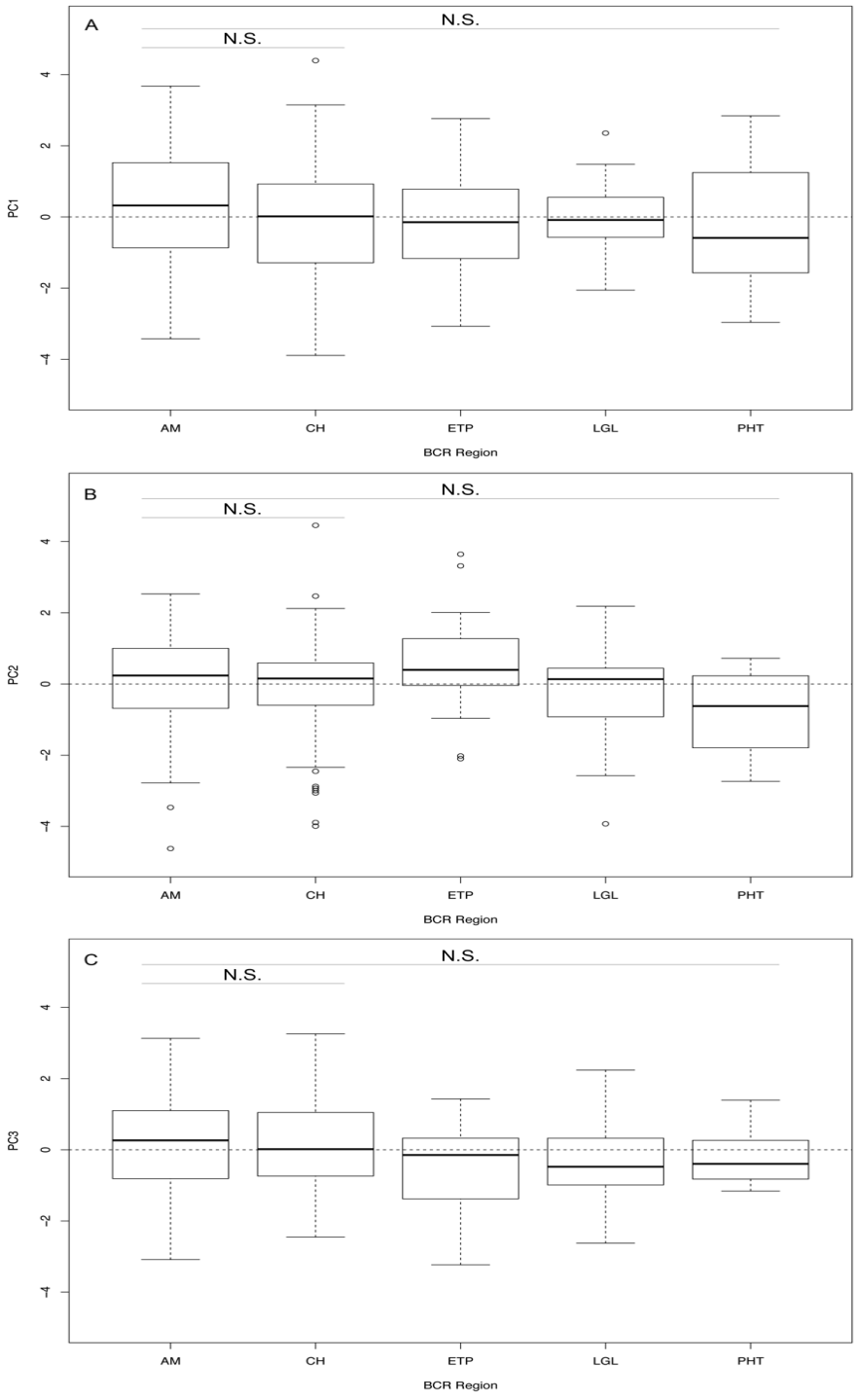

Figure 3. Boxplots showing Bird Conservation Region (BCR) data for principal components (PCs) 1-3 (A-C) for the 'wholesong' of the Cerulean Warbler. AM = Appalachian Mountains, CH = Central Hardwoods, ETP = Eastern Tallgrass Prairie, LGL = Lower Great Lakes, PHT = Prairie Hardwood Transition. All between-group differences were non-significant (N.S.).

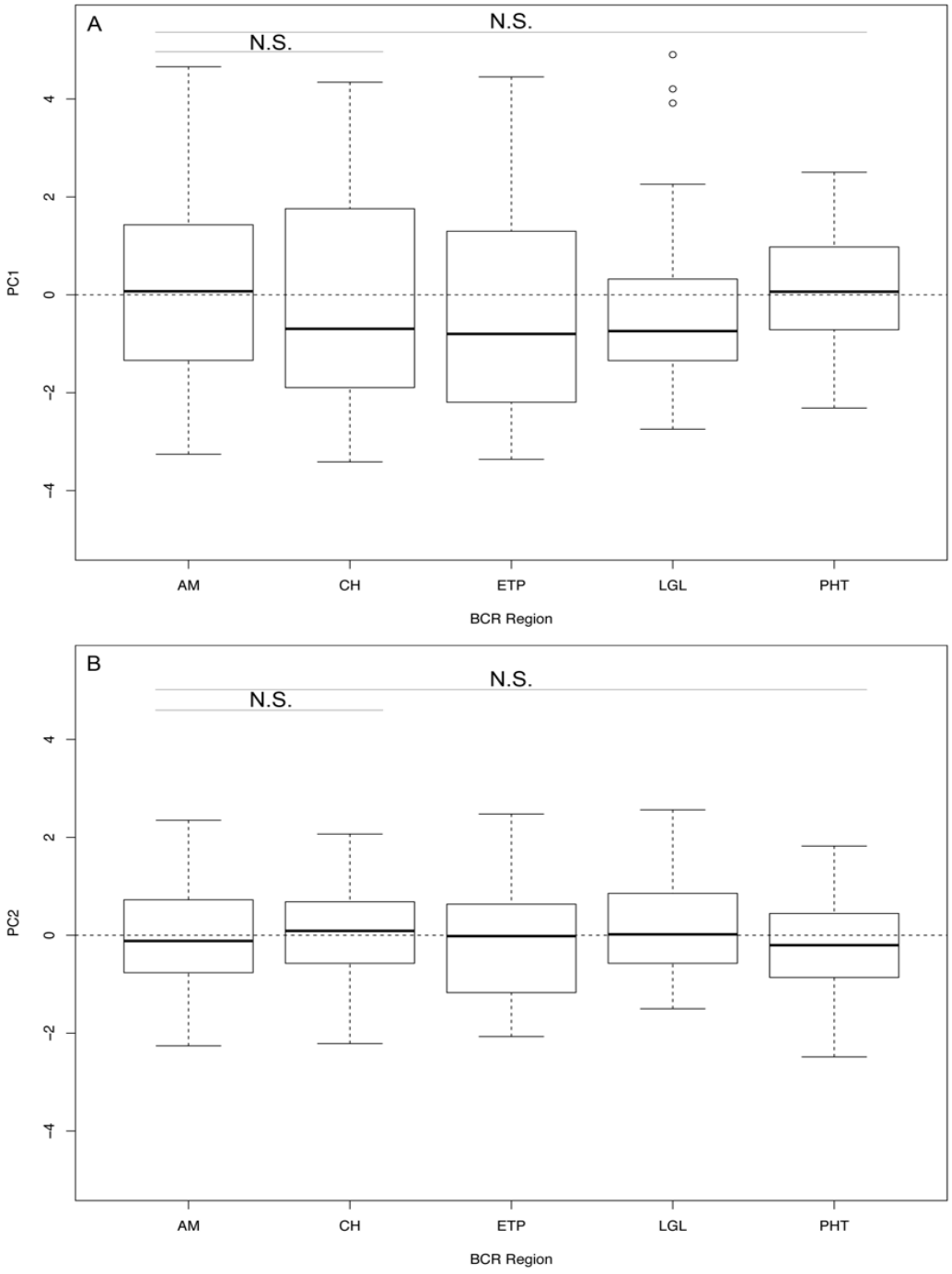

Figure 4. Boxplots showing Bird Conservation Region (BCR) data for principal components (PCs) 1-2 (A-B) for section 'A' of the Cerulean Warbler. AM = Appalachian Mountains, CH = Central Hardwoods, ETP = Eastern Tallgrass Prairie, LGL = Lower Great Lakes, PHT = Prairie Hardwood Transition. All between-group differences were non-significant (N.S.).

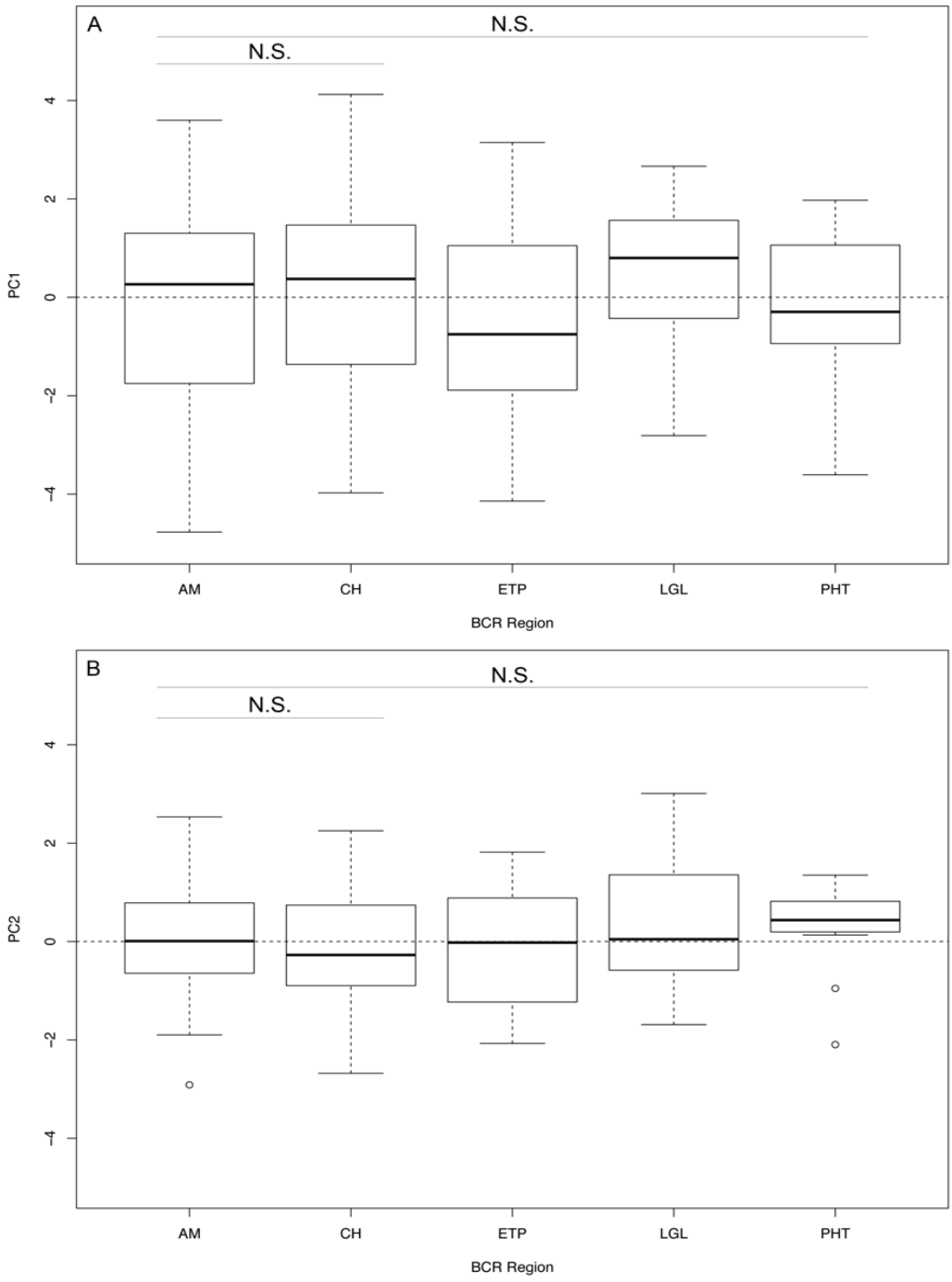

Figure 5. Boxplots showing Bird Conservation Region (BCR) data for principal components (PCs) 1-2 (A-B) for section 'B' of the Cerulean Warbler. AM = Appalachian Mountains, CH = Central Hardwoods, ETP = Eastern Tallgrass Prairie, LGL = Lower Great Lakes, PHT = Prairie Hardwood Transition. All between-group differences were non-significant (N.S.).

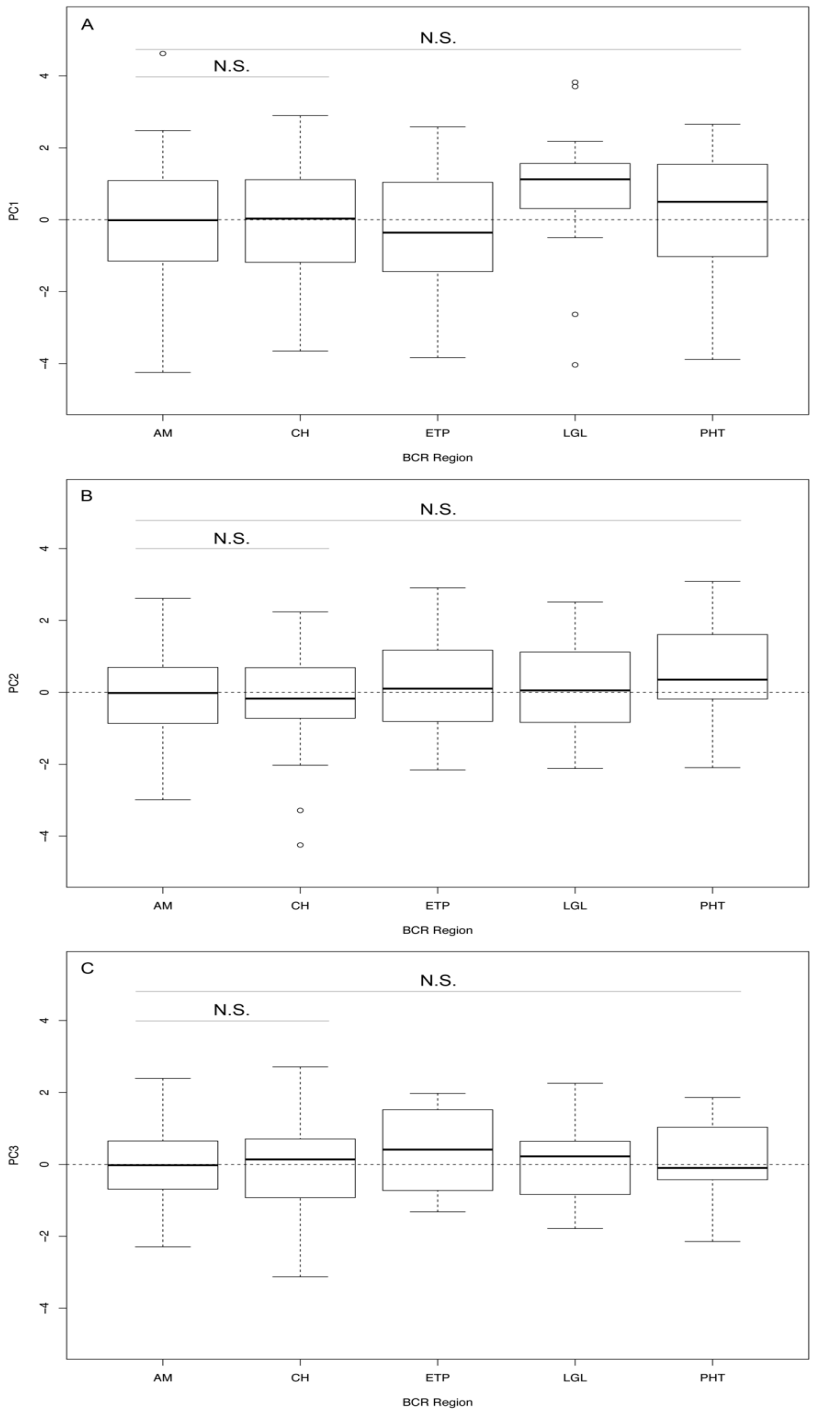

Figure 6. Boxplots showing Bird Conservation Region (BCR) data for principal components (PCs) 1-3 (A-C) for section 'C' of the Cerulean Warbler. AM = Appalachian Mountains, CH = Central Hardwoods, ETP = Eastern Tallgrass Prairie, LGL = Lower Great Lakes, PHT = Prairie Hardwood Transition. All between-group differences were non-significant (N.S.).

CHAPTER 1 TABLES

Table 1. Eigenvalues, standard deviation, and variance explained by principal components (PCs) of acoustic variables measured for Cerulean Warbler 'wholesongs.'

	Principal components		
	PC1	PC2	PC3
Eigenvalues	2.400	1.932	1.367
Standard deviation	1.549	1.390	1.169
Proportion of variance	0.300	0.242	0.171
Cumulative proportion of variance	0.300	0.542	0.713
Variable loadings			
Duration	0.591	-0.119	0.091
90^{th} Duration	0.559	-0.025	0.143
Center frequency	-0.003	0.544	-0.065
90^{th} Bandwidth	0.148	0.341	-0.625
Maximum frequency	0.080	0.571	-0.149
Minimum frequency	-0.137	0.433	0.506
Number of section types	0.308	-0.133	-0.401
Number of sections	0.443	0.204	0.370

Table 2. Eigenvalues, standard deviation, and variance explained by principal components (PCs) of acoustic variables measured for Cerulean Warbler song section 'A.'

	Principal components	
	PC1	PC2
Eigenvalues	3.968	1.091
Standard deviation	1.992	1.045
Proportion of variance	0.661	0.182
Cumulative proportion of variance	0.661	0.843
Variable loadings		
Duration	0.384	0.591
90^{th} Duration	0.388	0.587
Center frequency	-0.438	0.301
90^{th} Bandwidth	-0.378	0.305
Maximum frequency	-0.455	0.300
Minimum frequency	-0.400	0.178

Table 3. Eigenvalues, standard deviation, and variance explained by principal components (PCs) of acoustic variables measured for Cerulean Warbler song section 'B.'

	Principal components	
	PC1	PC2
Eigenvalues	3.783	1.295
Standard deviation	1.945	1.138
Proportion of variance	0.631	0.216
Cumulative proportion of variance	0.631	0.846
Variable loadings		
Duration	-0.449	0.256
90^{th} Duration	-0.376	0.428
Center frequency	0.446	0.136
90^{th} Bandwidth	0.161	0.781
Maximum frequency	0.453	0.330
Minimum frequency	0.476	-0.124

Table 4. Eigenvalues, standard deviation, and variance explained by principal components (PCs) of acoustic variables measured for Cerulean Warbler song section 'C.'

	Principal components		
	PC1	PC2	PC3
Eigenvalues	2.580	1.497	1.273
Standard deviation	1.606	1.224	1.128
Proportion of variance	0.430	0.250	0.212
Cumulative proportion of variance	0.430	0.679	0.892
Variable loadings			
Duration	-0.488	0.444	-0.235
90^{th} Duration	-0.482	0.445	-0.245
Center frequency	0.443	0.360	-0.265
90^{th} Bandwidth	0.143	0.406	0.693
Maximum frequency	0.400	0.557	0.027
Minimum frequency	0.391	-0.023	-0.578

Table 5. Percentages of 'wholesongs' and song sections 'A,' 'B,' and 'C' of Cerulean Warbler song that were correctly classified to the five Bird Conservation Regions by linear discriminant analysis.

	'Wholesongs'	Section 'A'	Section 'B'	Section 'C'
Appalachian Mountains	39.8	41.0	33.3	86.7
Central Hardwoods	62.1	60.5	68.8	0.0
Eastern Tallgrass Prairie	4.3	0.0	0.0	0.0
Lower Great Lakes	0.0	0.0	0.0	0.0
Prairie Hardwood Transition	0.0	0.0	0.0	0.0
Overall accuracy	37.3%	36.9%	39.4%	32.8%

Table 6. Percentages of 'wholesongs' and song sections 'A,' 'B,' and 'C' of Cerulean Warbler song that were correctly classified to the two Bird Conservation Regions by linear discriminant analysis.

	'Wholesongs'	Section 'A'	Section 'B'	Section 'C'
Appalachian Mountains	38.6	39.8	36.2	86.7
Central Hardwoods	62.1	60.5	65.0	0.0
Overall accuracy	50.6%	50.3%	51.7%	45.8%

CHAPTER 2: Intrinsic factors explain seasonal variation in dawn song characteristics of the Cerulean Warbler (*Setophaga cerulea*)

1. ABSTRACT

Male birds of many species sing prolifically during the 60 minutes before sunrise in a behavior known as "dawn song." Song rates during this time are generally much higher than during daytime singing, and the songs used during dawn song may differ from those used during the day. Despite an abundance of research on this behavior, its function is poorly understood. We studied the dawn singing behavior of a population of Cerulean Warblers (*Setophaga cerulea*) in south-central Indiana from May to July 2017. We recorded 100 dawn song performances from 12 paired male Cerulean Warblers using autonomous recording units, and we determined the relative influences of seasonality, breeding stage, and weather on six characteristics of dawn singing behavior. Seasonality (Julian date) strongly governed the presence of dawn song such that males were more likely to sing early in the season rather than late; indeed, dawn song mostly ceased by the middle of the breeding season (mid-June). Dawn song start times in relation to sunrise varied little over the season, but males ended dawn singing earlier as the season progressed, such that bout length decreased from May to July. The peak dawn song rate peaked early in the season and declined sharply with Julian date. Additionally, males initiated and ended dawn song bouts later on mornings that were windy and rainy. This is the first comprehensive study of dawn song in the Cerulean Warbler. The breeding stage of the pair's nest was not as important at predicting dawn singing behavior as in other wood-warblers. Our results indicate that dawn singing in this species may be governed primarily by intrinsic factors (hormones) rather than by social factors (i.e., as a response to the female fertile period).

Keywords: birdsong, Cerulean Warbler, dawn song, *Setophaga cerulea*

2. INTRODUCTION

During the breeding season, many male birds participate in an intense bout of community-wide singing during the hour before sunrise (e.g., the "dawn chorus"), which is known as "dawn song" (Catchpole and Slater 2008). Dawn singing is a widespread behavior in avian species, both in oscines and suboscines, and in temperate and tropical systems (reviewed in Staicer et al. 1996). Song rates during the dawn bout are typically much higher than later in the day, and the songs that males use for dawn singing are often different than those used for daytime singing, particularly in species with small song repertoires (e.g., Spector 1992, Woodward 1997, Zhang et al. 2015). As sunrise approaches, dawn song rates typically decrease and eventually cease, followed by a period of silence (the "sunrise lull") and the commencement of daily activities (e.g., foraging, daytime singing; Kroodsma 1971, Liu and Kroodsma 2007). Despite fascinating researchers for decades (e.g., Allard 1930, Nice 1943, Leopold and Eynon 1961), dawn song is still poorly understood and understudied, particularly compared to daytime singing behaviors. In addition, dawn and daytime songs may have different functions, and dawn song may play an adaptive role in both inter- and intrasexual communication.

The primary hypotheses proposed to explain the functions of dawn song can be classified into three categories, and they include both proximate (environmental and intrinsic) and ultimate (social) explanations (reviewed in Staicer et al. 1996). Males may sing at dawn because that is the time of day when sound transmission is optimal. Certain atmospheric conditions that are common at dawn (e.g., low wind, high humidity) may be more conducive for efficient song propagation; this idea is known as the "acoustic adaptation hypothesis" (Morton 1975, Henwood

and Fabrick 1979). Dawn song may also be a response to unprofitable foraging conditions during the hour before sunrise when light levels are low and foraging efficiency is reduced (e.g., Kacelnik and Krebs 1982), though some studies indicate that invertebrate activity may not be reduced in the morning (e.g., Mace 1987). In contrast, males may sing at dawn as a consequence of circadian patterns of elevated levels of circulating hormones (i.e., testosterone). For example, seasonal variation in singing is correlated with plasma testosterone levels in the Willow Tit (*Poecile montanus*; Rost 1992). However, there are numerous examples where hormones and song production in avian species are not so tightly linked (e.g., Nowicki and Ball 1989, Wingfield and Hahn 1994).

Dawn song may also have a number of ultimate explanations related to social functions. It may play a role in mate attraction (deVos 1983, Merritt 1985), mate stimulation (e.g., Wingfield and Farner 1993), mate guarding (Mace 1986), territorial defense (Amrhein and Erne 2006), and adjustment of social relationships with neighboring males (e.g., Burt and Vehrencamp 2005). In some species, dawn singing behavior is correlated with the female fertile period (e.g., Eastern Phoebe *Sayornis phoebe* Bruni and Foote 2014; Willow Tit *Poecile montanus* Welling et al. 1995), when territorial intrusions from neighboring males seeking extra-pair copulations may be most likely (e.g., Kacelnik and Krebs 1983, Johnson and Kermott 1989). Hypotheses include both inter- and intrasexual functions, and explanations are not necessarily mutually exclusive; dawn song may function in a way that targets both sexes (Kroodsma et al. 1989, Burt and Vehrencamp 2005). Thus, the function of dawn song is still unclear, but both biotic and abiotic factors likely drive patterns in dawn singing behaviors.

Studies of dawn song in wood-warbler species (family Parulidae) suggest that social factors may best explain the function of dawn song. In most wood-warblers, the songs used by

males during the dawn chorus tend to be different than those used later in the day (Staicer et al. 1996). Specifically, most wood-warblers with a Type I/Type II song system use their Type II songs (hypothesized to target males) during the dawn bout (Staicer et al. 1996). Interestingly, dawn song tends to be absent early in the season (before females have arrived to the breeding grounds or during early male territory establishment), indicating that dawn song may function intersexually or that its initiation only occurs after competition for and defense of territories is high (Highsmith 1989, Spector 1991). For example, Golden-cheeked Warblers (*Setophaga chrysoparia*) do not sing dawn song early in the season just after their arrival to the breeding grounds, but once dawn singing behavior is initiated, males tend to sing from territory edges near adjacent singing males (Bolsinger 2000). Previous research on wood-warblers has found that males tend to sing dawn songs throughout the nesting period, with a peak in bout length after the female's clutch is laid (Kroodsma et al. 1989, Spector 1991, Woodward 1997). This may indicate a territorial defense function during this period of increased pressure from rival males seeking extra-pair copulations. Thus, in the wood-warblers, dawn song may function primarily for territorial defense, though other factors may play a role.

We used autonomous recording units to study the dawn singing behavior of the Cerulean Warbler (*Setophaga cerulea*) in south-central Indiana, USA. The Cerulean Warbler is an oscine passerine with learned songs (Buehler et al. 2013). Males have a repertoire of two to three song types that can be separated based on differences in the contexts in which each is used: Type I songs are used during the day for mate attraction, while Type II songs are used for dawn singing and are thought to function in territorial defense (Woodward 1997, GJ MacDonald pers. ob.). Wood-warblers that are taxonomically related often share many of the characteristics of their singing behaviors, so we hypothesized that Cerulean Warbler dawn song would be similar to that

of other *Setophaga* warblers. The objective of our research was to investigate intrinsic, environmental, and social explanations of dawn song in paired male Cerulean Warblers. We determined the relative influence of the breeding stage of the pair's nest, seasonality (Julian date), and weather (temperature, wind, and rain) on six characteristics of dawn song. Our study is the first comprehensive exploration of dawn singing behavior in this species.

3. METHODS

3.1 Field Methods

We recorded 100 dawn song performances from 12 paired male Cerulean Warblers from 12 May to 3 July 2017. Fieldwork occurred in Yellowwood and Morgan-Monroe state forests in Morgan, Monroe, and Brown counties in south-central Indiana, USA. Our research sites are part of the Hardwood Ecosystem Experiment, a long-term (100 years), multi-taxa, multi-organizational study initiated in 2006 investigating the effects of forest management on various taxa (Swihart et al. 2013). Nests were monitored for 30 min every one to three days until their completion (fledge or fail). We recorded nest status as build, lay, incubation, nestling, or fledging based on our estimation of nest status and adult behaviors (e.g., adult arrival at partial nest with nest material, arrival at nest with food in bill). Cerulean Warbler nest transition dates in our system are approximate (within ~ 2 days) because nests at our field sites are on average 18 m high in the canopy and their contents cannot be observed (Wagner and Islam 2014). For this study, we combined several breeding stage categories due to the imprecision of nest stage transition dates, which resulted in a three-category variable for breeding stage: build/lay, incubation, and nestling. In cases when we located nests after they had been built, we backdated and estimated breeding stages based on the known average length of each stage (Buehler et al.

2013). Pairs will make repeated nesting attempts (Buehler et al. 2013), and since not all attempts are discovered, we did not consider whether each nesting attempt was a first or second brood. The number of dawn song performances recorded per male varied from 3-17 (mean = 8.3, SD = 4.44). We collected dawn song recordings from all three breeding stages for two males, from two breeding stages for five males, and from only one breeding stage for five males.

We used autonomous recording units (hereafter, ARU; model SM4 Wildlife Acoustics, Inc., Concord, Massachusetts, USA) to record the dawn singing behavior of paired males. All recordings were made in WAV stereo format with 16-bit precision and a sampling rate of 44.1 kHz. We deployed ARUs within ten vertical meters and 15 horizontal meters of each nest using a canopy deployment system that utilizes a bow and arrow, ¼ inch rope, braided fishing line, and carabiners. Each ARU was programmed to recognize local sunrise using a Global Positioning System (GPS), and each unit was scheduled to record from 60 minutes before sunrise until 1300 hours (recording end time was based on a protocol from a separate project). We made a concerted effort to record dawn song from a portion of each nest's breeding stage (i.e., build/lay, incubation, or nestling). We rotated the placement of ARUs such that each unit recorded for approximately three to seven days before being moved to a different nest; thus, most males had incomplete coverage of the three breeding stages comprising their nesting attempt. On average, males began singing 37 min before sunrise (range 24 to 46 min), and males ended dawn song bouts 12 min on average before sunrise (range 22 min before sunrise to 17 min after). Thus, we are confident we captured the entire dawn bout with our automated recording protocol. Civil twilight and sunrise times were determined using Time and Date AS (Time and Date AS 2018).

3.2 Audio Recording Analysis

We visualized spectrograms in Raven Pro 1.5 using a Hann window, FFT = 512, and 50% overlap (Bioacoustics Research Program 2014). We used the spectrogram cursor to mark instances of dawn song from the time of first song until the male stopped singing. The end of the dawn song bout was defined as the point when males either stopped singing for three minutes or switched from dawn to daytime singing behaviors (i.e., a sudden change in song types corresponding to a switch from Type I to Type II singing behaviors; Woodward 1997). We calculated six characteristics of each dawn song bout: 1) if the male sang dawn song or not (binary: yes = 1, no = 0), 2) length of dawn bout (min), 3) time before sunrise of first song, 4) time in relation to sunrise of last song, 5) average song rate (songs/min), and 6) peak song rate (songs/min; Table 1). Average song rate was the mean of the per minute song rates over the course of the dawn song bout. Peak song rate was the maximum number of songs per minute during the dawn bout.

3.3 Statistical Analysis

We used generalized linear mixed models (GLMM) to analyze how seasonality, breeding stage, and weather influenced the dawn singing behavior of paired male Cerulean Warblers. Seasonality was the Julian date of each audio recording, breeding stage was a three-category variable representing discrete portions of the species' nesting cycle (build/lay, incubation, and nestling), and weather included temperature (°C), wind (mph), and the presence of rain (binary: yes = 1, no = 0), for a total of five predictors (Table 2). Weather data came from the Bloomington Monroe County Airport (39.1333°, -86.6167° W) and corresponded to the hour nearest the start of dawn song for which data were available. "Individual" (male identity) was included as a random effect in all of our models to account for repeated measures of individuals

and individual variation in dawn singing behavior. The most parsimonious models were selected based on Akaike's Information Criterion corrected for small sample sizes (AICc). All models with ΔAICc values of < 2.0 were considered equally plausible (Burnham and Anderson 2002). All statistical analyses were carried out in Program R (R Development Core Team 2016) with the lme4 (Bates et al. 2015) and bbmle (Bolker and R Development Core Team 2017) packages.

We performed model averaging using the MuMIn package (Bárton 2018) when more than one model was selected based on AICc. We used model-averaged coefficients to generate predictions of dawn singing behavior based on each covariate in the candidate models.

4. RESULTS

4.1 Dawn Song Presence

The presence of dawn song was best explained by seasonality and weather (Table 3). Julian date was the most important covariate, appearing in all three models with ΔAICc values of < 2.0 (Table 4). Temperature and wind were also included in one model each. Paired male Cerulean Warblers were more likely to sing early rather than late in the breeding season (-2.85, [95% CI = -4.97, -0.73]; Figure 1). Also, males were less likely to sing on mornings that were warm (-0.14, [95% CI = -1.33, 0.38]; Figure 2) and windy (-0.10, [95% CI = -1.15, 0.40]; Figure 3).

4.2 Bout Length

Analysis of the length of the dawn song bout produced two equally plausible models with ΔAICc values of < 2.0 (Table 5). Julian date and breeding stage were included in both models, while rain occurred in one model (Table 6). Bout length decreased as the breeding season

progressed (-1.32, [95% CI = -1.91, -0.74]; Figure 4). Bout lengths during incubation decreased with advancing Julian date (-0.08, [95% CI = -0.70, 0.53]; Figure 5). Males increased bout lengths during the nestling period (1.06, [95% CI = 0.05, 2.07]; Figure 5), though this relationship may have been driven by a small sample size of late season dawn bouts during the nestling stage. Additionally, males sang shorter bouts on rainy mornings (-0.39, [95% CI = -1.51, 0.07]; Figure 6).

4.3 Start of Dawn Song

The start time of dawn singing was largely driven by weather (Table 7). Rain was included in all four models, while temperature and wind were included in two models each (Table 8). Paired male Cerulean Warblers began dawn song bouts later when it was raining (-0.14, [95% CI = -0.26, -0.03]; Figure 7). Additionally, males initiated dawn song later in the day on warm mornings (-0.01, [95% CI = -0.05, 0.02]; Figure 8) and when it was windy (-0.02, [95% CI = -0.06, 0.005]; Figure 9).

4.4 End of Dawn Song

Analysis of the factors influencing the end time of the dawn song bout resulted in four equally plausible models with ΔAICc values of < 2.0 (Table 9). Julian date, breeding stage, and rain were included in all of the models (Table 10), while temperature and wind occurred in two models each (Table 10). Males ended dawn song bouts later, and after sunrise, early in the breeding season (-13.27, [95% CI = -18.28, -8.27]; Figure 10). Dawn bout end times during incubation decreased with advancing Julian date (0.94, [95% CI = -6.44, 8.32]; Figure 11). However, end times during the nestling period were closer to sunrise such that bouts were longer

(7.02, [95% CI = -3.40, 17.43]; Figure 11). Males ended dawn bouts later on mornings that were rainy (0.44, [95% CI = -10.70, 11.60]; Figure 12) and warm (0.47, [95% CI = -2.46, 4.22]; Figure 13), but stopped singing earlier during windy weather (-0.51, [95% CI = -4.42, 2.53]; Figure 14).

4.5 Average Song Rate

Analysis of average song rate produced six models with ΔAICc values of < 2.0 (Table 11), including the null model (random effect of individual). Average song rate was not explained by seasonality, breeding stage, or weather (Table 11). Individual variation had as much explanatory power as did any of the predictors.

4.6 Peak Song Rate

Seasonality largely explained peak song rate during the dawn song bout, occurring in all five models with ΔAICc values of < 2.0 (Table 12). Breeding stage occurred in three models, rain in two models, and temperature in one model (Table 13). Males had a lower peak song rate later in the season (-3.18, [95% CI = -5.20, -1.16]; Figure 15). Peak song rate was high early in the season during incubation, but was negatively related with Julian date (-0.01, [95% CI = -1.68, 1.64]; Figure 16). Peak song rate increased during the nestling period, but the relationship was still negative throughout the season (1.32, [95% CI = -1.02, 5.07]; Figure 16). Males also had lower peak song rates when it rained (-0.11, [95% CI = -2.36, 1.79]; Figure 17) and when temperatures were warmer (0.03, [95% CI = -0.31, 0.89]; Figure 18), though these relationships were weak.

5. DISCUSSION

Dawn singing behavior in paired male Cerulean Warblers was largely driven by seasonality (Julian date). Males were more likely to sing dawn song early in the breeding season rather than late, and they also sang longer bouts, and at faster peak rates early in the season. Indeed, all males sampled from 12 May to 11 June sang dawn song (45 of 51 possible dawn song recording events) while only three of the five males sampled during the late season sang dawn song (defined here as 12 June-3 July; nine of 49 possible dawn song recording events). Most dawn song performances were initiated before the start of civil twilight (the time when the sun is 6° below the horizon), and the start time in relation to sunrise varied minimally throughout the season. Bout lengths decreased as the season progressed such that dawn song bouts ended sooner, and before sunrise, as the season advanced. Additionally, peak song rates were higher early in the season and decreased steadily with Julian date.

Interestingly, we found that dawn bout lengths during build/lay and incubation were similar, but that males lengthened their bouts during the nestling stage. This finding is inconsistent with the mate-guarding hypothesis for the function of dawn song, which predicts that dawn song bouts will be longest during the fertile period of the female (pre-lay and early-lay) in order to deter rival males from soliciting extra-pair copulations (Mace 1986). The increase in dawn song rates during the nestling period is consistent with other research showing an increase in wood-warbler dawn song bout length after the male's mate lays a clutch (Highsmith 1989, Staicer 1991). The function of a longer dawn song bout after the clutch is unclear, though it could be the result of increased testosterone levels. For instance, male testosterone levels might peak during this energetically-demanding time, when males often feed the incubating female and are responsible for about half of the responsibilities of feeding nestlings (Buehler et al. 2013, GJ MacDonald pers. obs.). It should be noted, however, that very

little is known about how testosterone levels vary seasonally in many species of wood-warblers, including the Cerulean Warbler.

Despite the strong effect of seasonality in our models, weather also explained dawn singing behaviors. We found that males delayed the start of dawn song during inclement weather; specifically, males started singing later when it was rainy and windy. Males were also less likely to sing on mornings that were warm and windy, they ended dawn bouts later on mornings that were rainy and warm, and they stopped singing earlier during windy weather. Rainy and windy days were also characterized by having lower peak song rates. These findings are consistent with other studies that have found that weather affects dawn song (e.g., Allard 1930). For example, Yellow Warblers (*Setophaga petechia*) delayed dawn song on days that began cold and wet (Spector 1991), presumably because rain is correlated with cloud cover, which is known to delay the dawn chorus start times of temperate-breeding species (Bruni 2013). Additionally, rain is known to cause a decrease in animal activity (Link et al. 2011), though the effects of rain on the singing behavior of birds has rarely been quantified. The result that temperature affects the presence, end time, and peak song rate of dawn song contrasts with the few previous studies of the effects of ambient temperature on dawn singing behavior (e.g., Bruni 2013). We believe this pattern may have been driven by the relationship between temperature and Julian date (i.e., ambient temperature increases from spring to summer) in our system rather than the existence of a direct link between temperature and dawn singing behavior.

Dawn song in paired male Cerulean Warblers was more strongly predicted by intrinsic factors (seasonality) rather than by the breeding stage of the pair's nest. Testosterone is known to cause increased singing and heightened responsiveness to stimuli, such as that caused by countersinging males. Additionally, male testosterone levels are thought to vary seasonally,

though data on potential correlations between testosterone and singing behavior in many bird species is scant (Wingfield and Farner 1993; reviewed by Staicer et al. 1996). Additionally, due to the height and inaccessibility of Cerulean Warbler nests in our system, our estimates of breeding stage transitions were only accurate to within a few days. Thus, it is possible that our breeding stage transitions lacked the precision necessary to accurately assess the influence of breeding stage on dawn song behavior. Despite the challenges presented considering the canopy nesting behavior of this species, future studies of Cerulean Warbler dawn song should attempt to develop methods to monitor the nests' contents and to more closely link circulating levels of testosterone with singing behavior.

Our results showed that dawn singing characteristics of paired male Cerulean Warblers were more strongly governed by seasonality than by breeding stage or weather. Males were more likely to sing early rather than late in the season and most males ceased singing dawn song by mid-June, approximately halfway through the local breeding season. Average and peak song rates were highest early in the season and declined noticeably as the season progressed. Additionally, we found that males delayed dawn song start times due to rainy and windy weather. Our results support intrinsic explanations for variation in dawn song performance. However, it is clear that explanations for the function of dawn song are complex and many are not mutually exclusive; thus, other explanations may also be supported by our data, including a territorial function.

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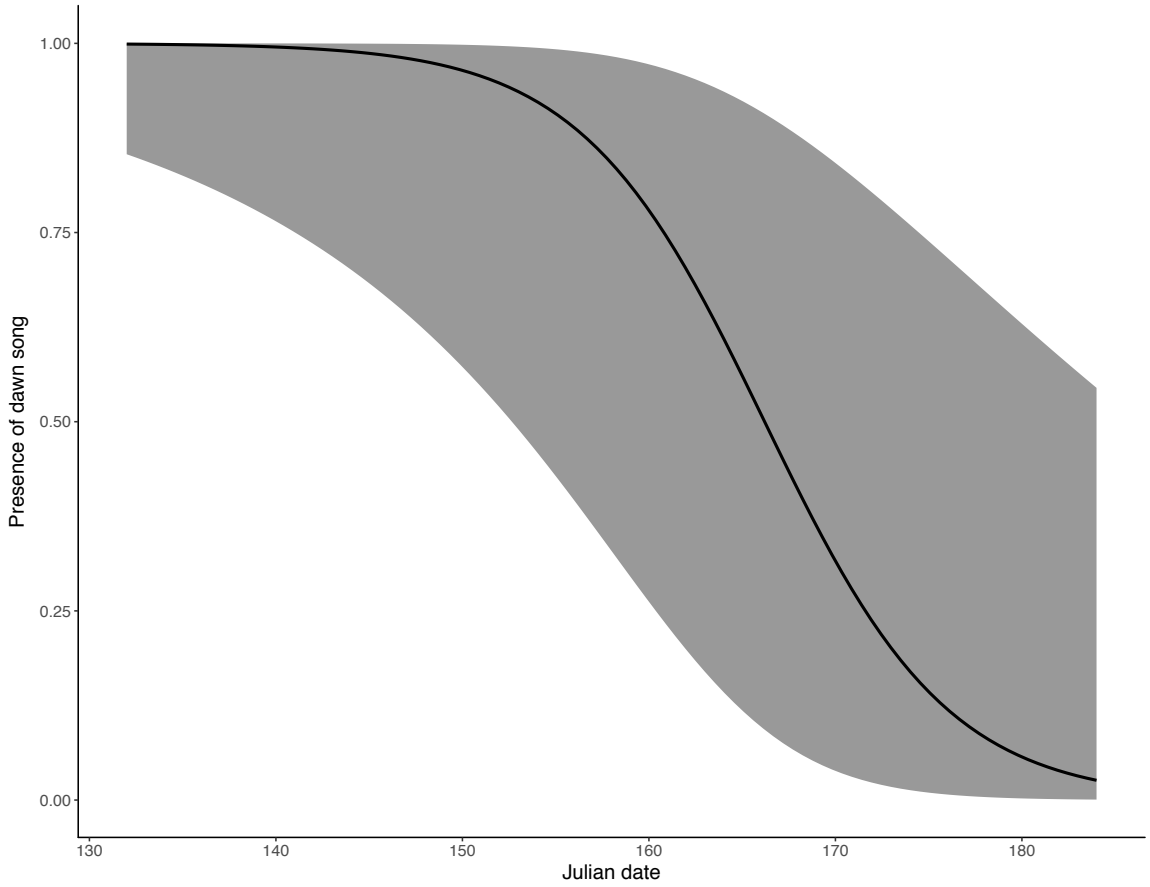

Figure 1. Model-averaged prediction for the influence of Julian date on the presence of dawn song by paired male Cerulean Warblers in 2017 in south-central Indiana. Males were more likely to sing dawn song early in the season (May and early June) rather than late in the season (late June and early July). The shaded gray area represents 95% confidence intervals.

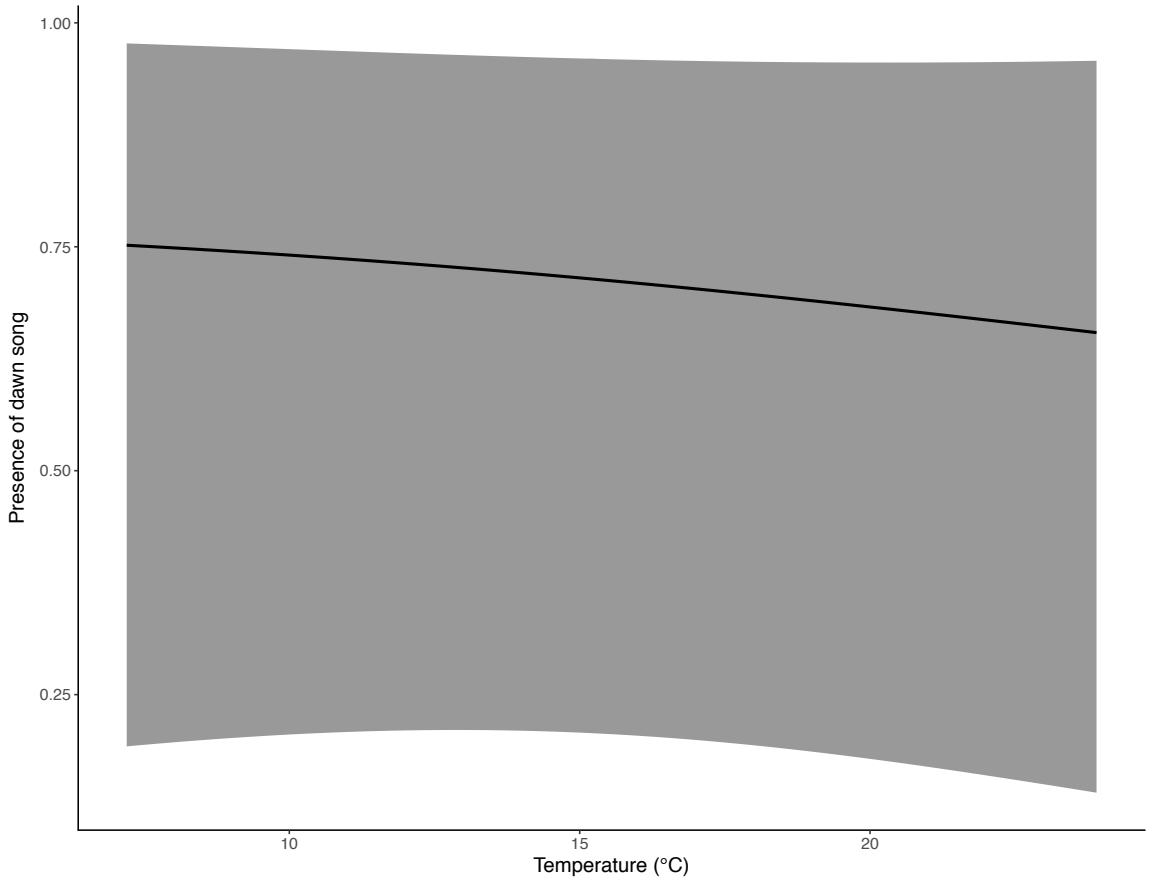

Figure 2. Model-averaged prediction for the influence of temperature (°C) on the presence of dawn song by paired male Cerulean Warblers in 2017 in south-central Indiana. Males were slightly less likely to sing dawn song on mornings that were warm. Shaded gray areas represent 95% confidence intervals.

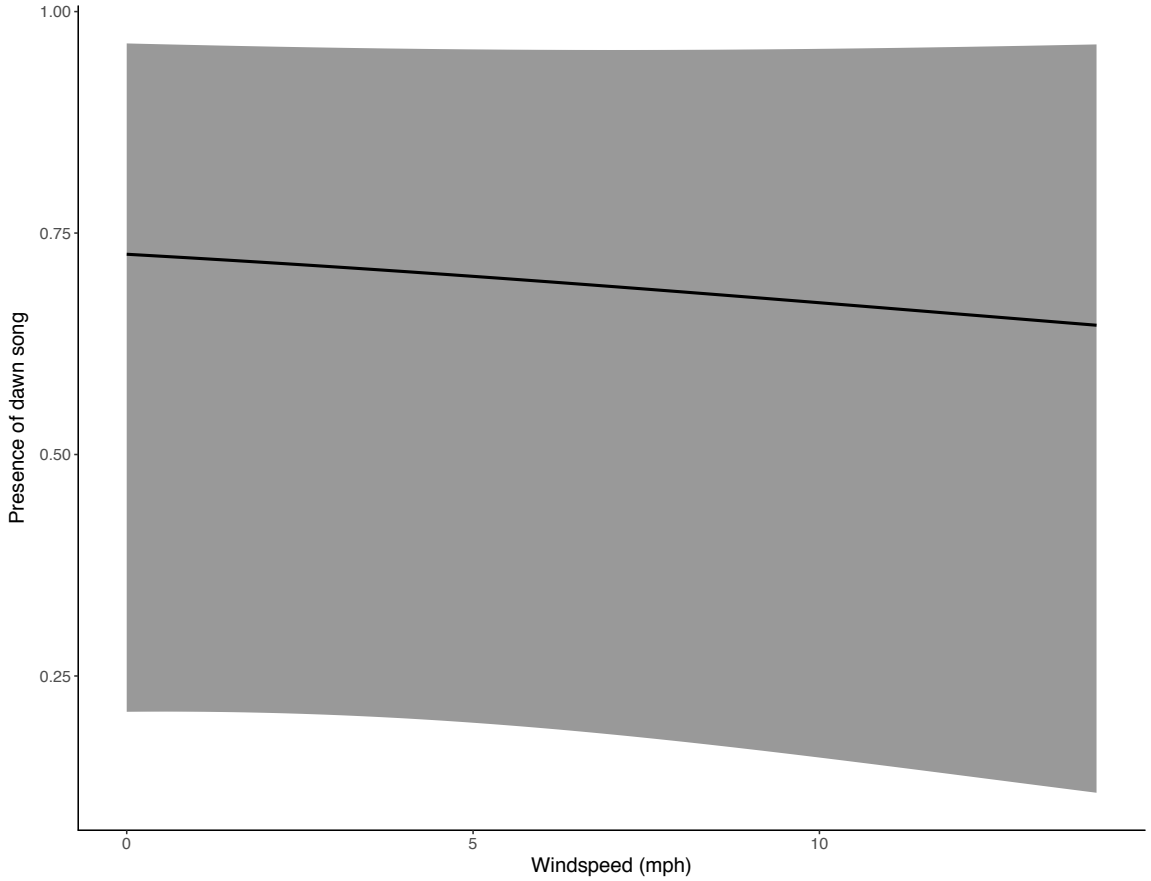

Figure 3. Model-averaged prediction for the influence of wind on the presence of dawn song by paired male Cerulean Warblers in 2017 in south-central Indiana. Males were slightly less likely to sing dawn song on windy mornings. Shaded gray areas represent 95% confidence intervals.

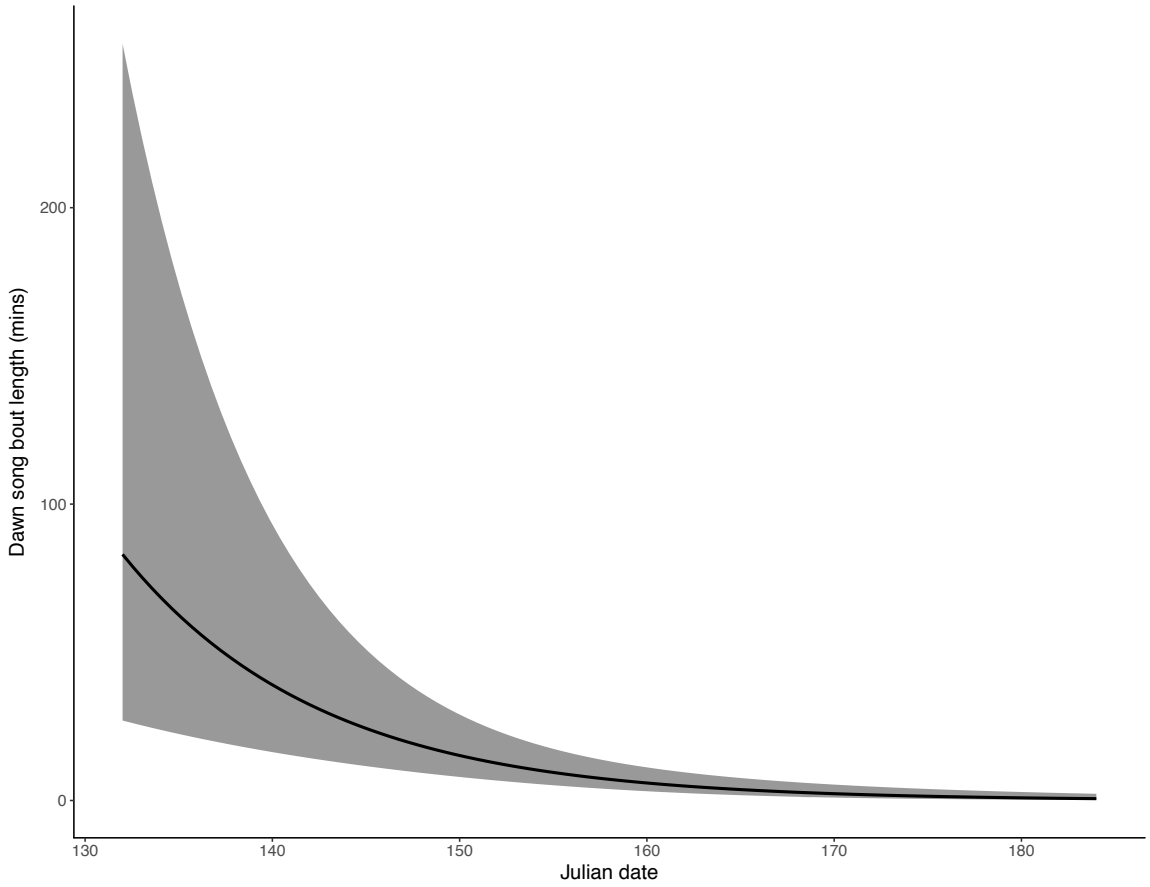

Figure 4. Model-averaged prediction for the influence of Julian date on the dawn song bout length of paired male Cerulean Warblers in 2017 in south-central Indiana. Bout lengths were much longer early in the season (May and early June) than they were later in the season (late June and early July). Shaded gray areas represent 95% confidence intervals.

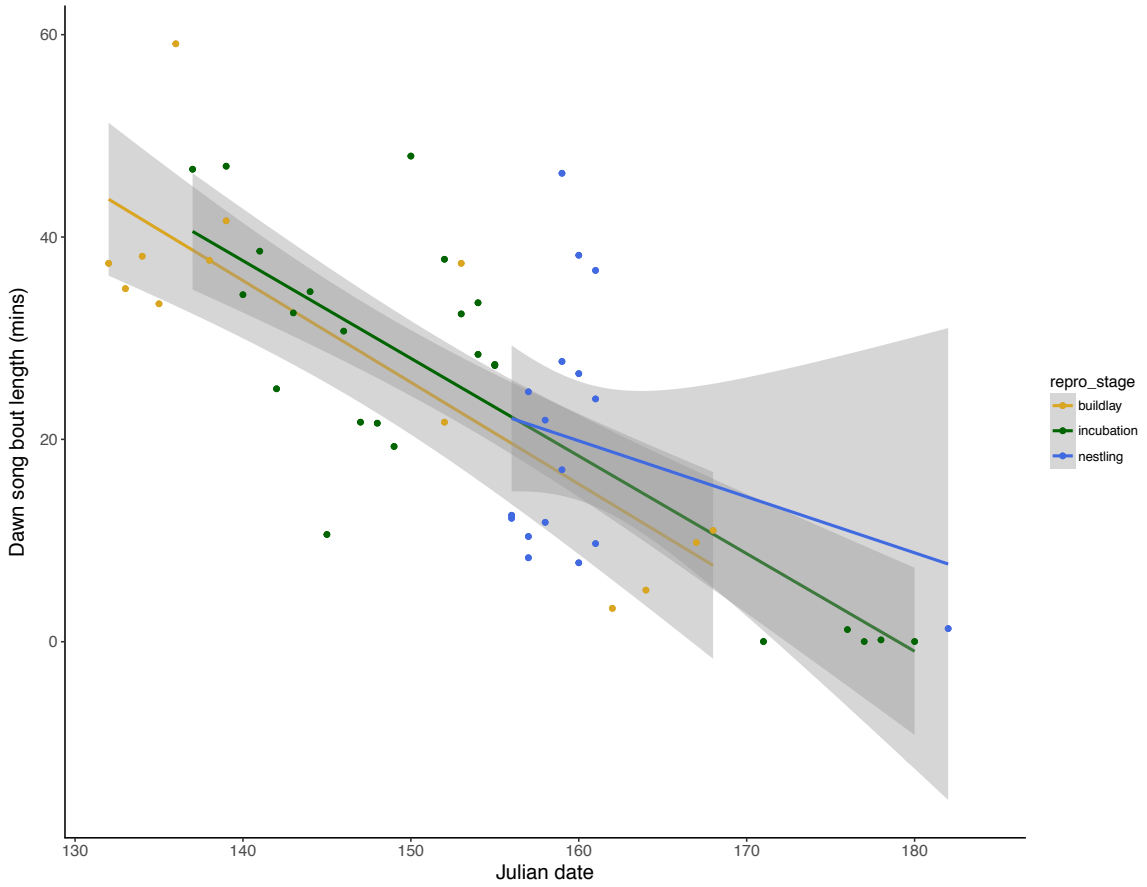

Figure 5. Model-averaged prediction for the influence of breeding stage (build/lay, incubation, nestling) on the dawn song bout length of paired male Cerulean Warblers in 2017 in south-central Indiana. Bout length decreased throughout the breeding season, regardless of breeding stage. Shaded gray areas represent 95% confidence intervals.

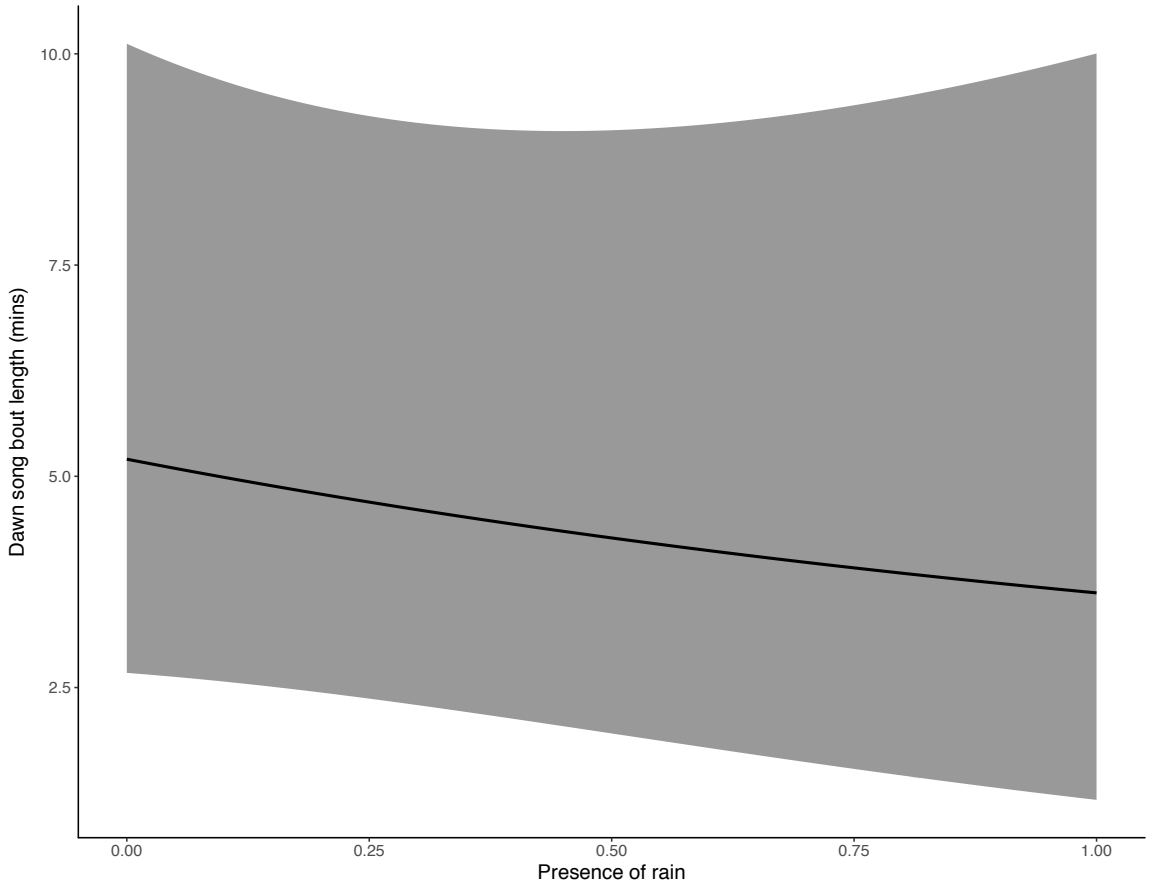

Figure 6. Model-averaged prediction for the influence of the presence of rain on the dawn song bout length of paired male Cerulean Warblers in 2017 in south-central Indiana. Bout length was negatively correlated with the presence of rain. Shaded gray areas represent 95% confidence intervals.

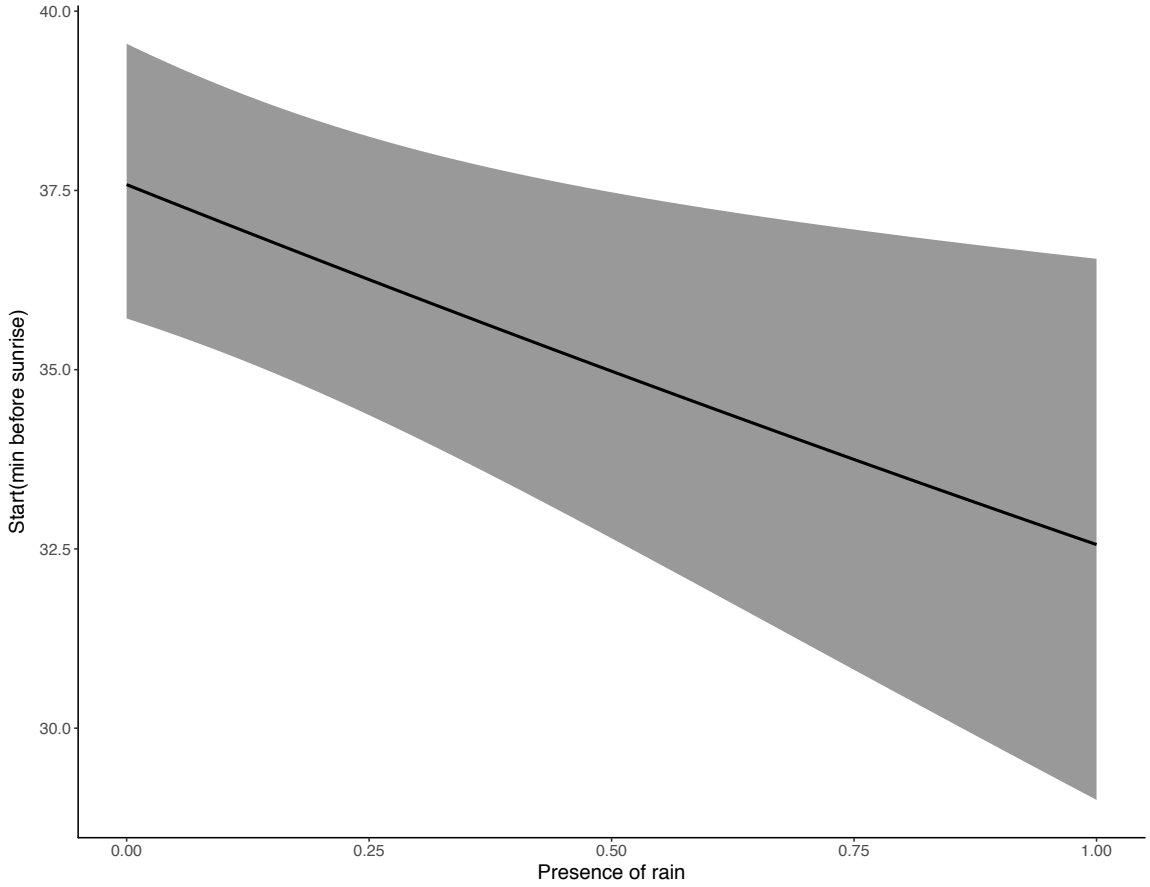

Figure 7. Model-averaged prediction for the influence of the presence of rain on the start time of the dawn song bout in relation to sunrise of paired male Cerulean Warblers in 2017 in south-central Indiana. Males initiated dawn song bouts later on mornings that were rainy. Shaded gray areas represent 95% confidence intervals.

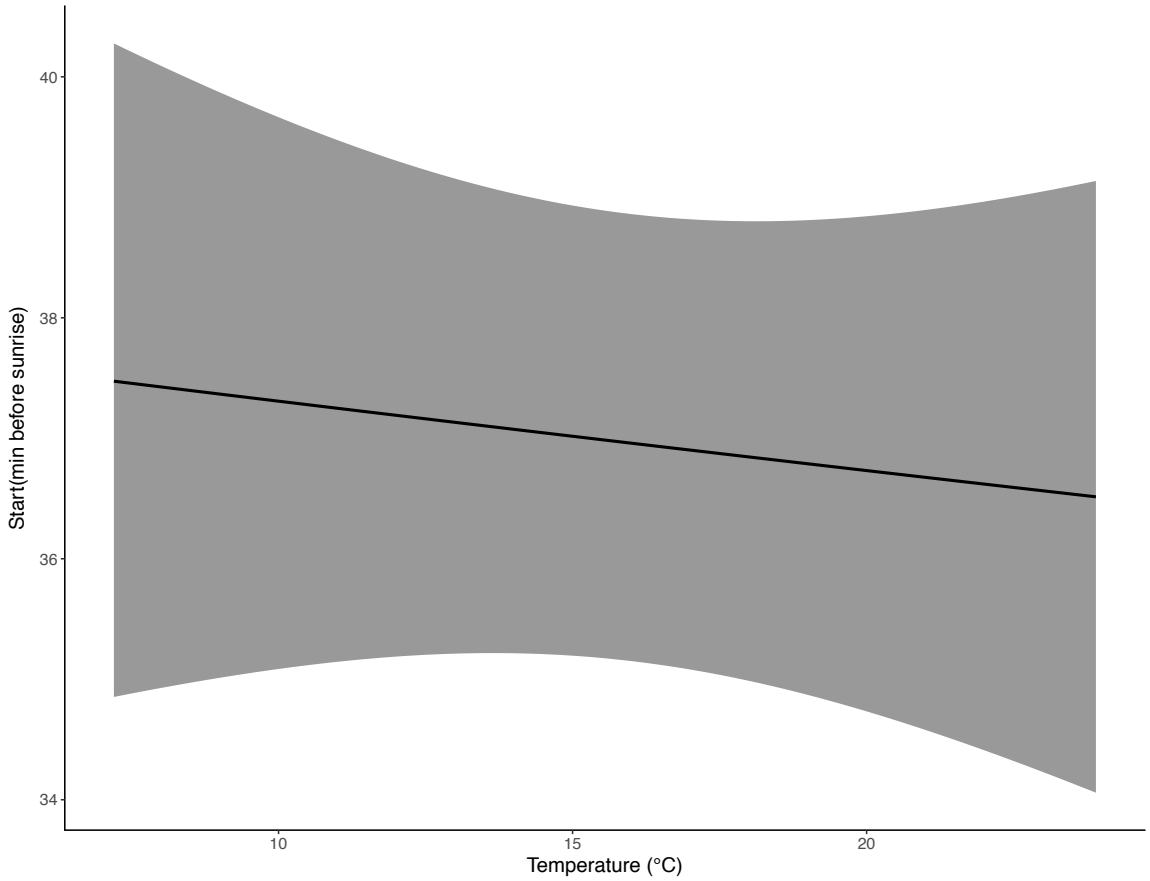

Figure 8. Model-averaged prediction for the influence of temperature (°C) on the start time of the dawn song bout in relation to sunrise of paired male Cerulean Warblers in 2017 in south-central Indiana. Males initiated dawn song bouts later on warm mornings. Shaded gray areas represent 95% confidence intervals.

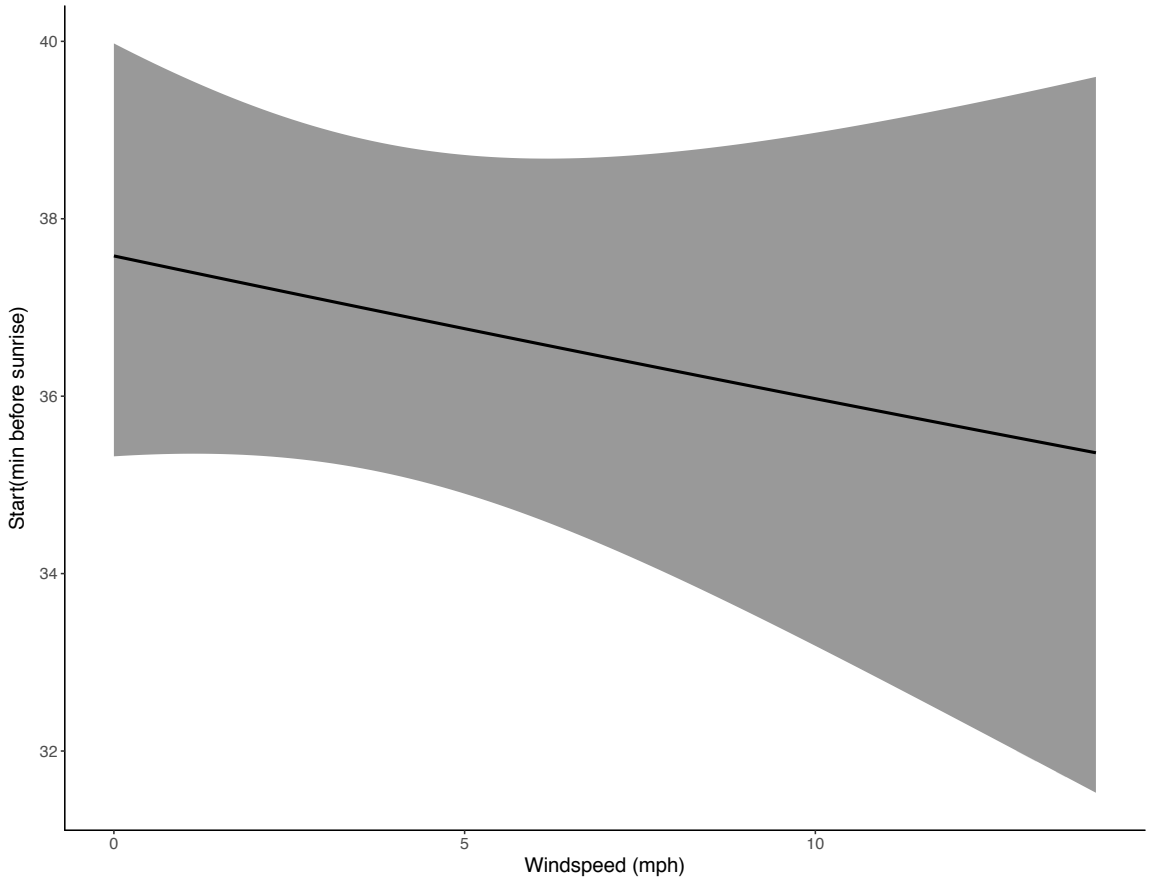

Figure 9. Model-averaged prediction for the influence of wind on the start time of the dawn song bout in relation to sunrise of paired male Cerulean Warblers in 2017 in south-central Indiana. Males initiated dawn song bouts later on mornings that were windy. Shaded gray areas represent 95% confidence intervals.

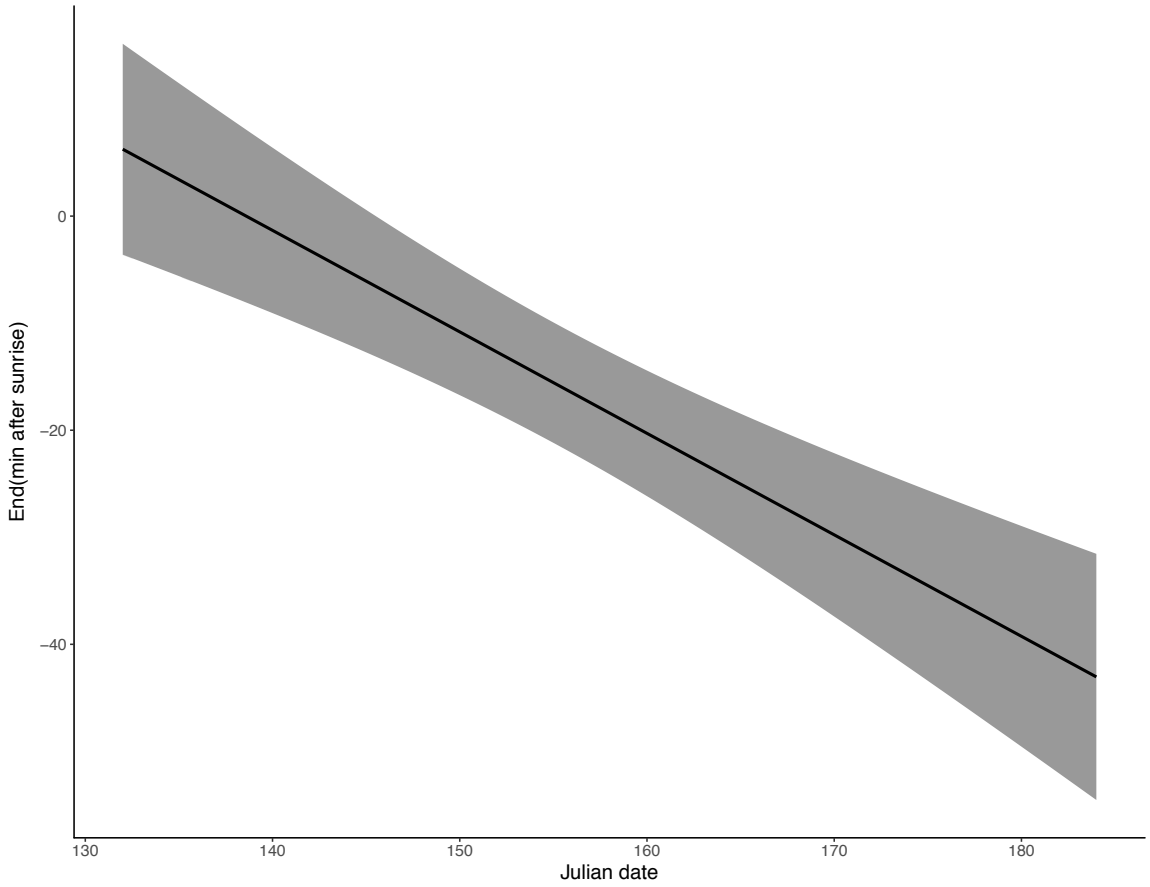

Figure 10. Model-averaged prediction for the influence of Julian date on the end time of the dawn song bout in relation to sunrise of paired male Cerulean Warblers in 2017 in south-central Indiana. Early in the season (May), males sang dawn song past sunrise, but they ended sooner in relation to sunrise as the season progressed. Shaded gray areas represent 95% confidence intervals.

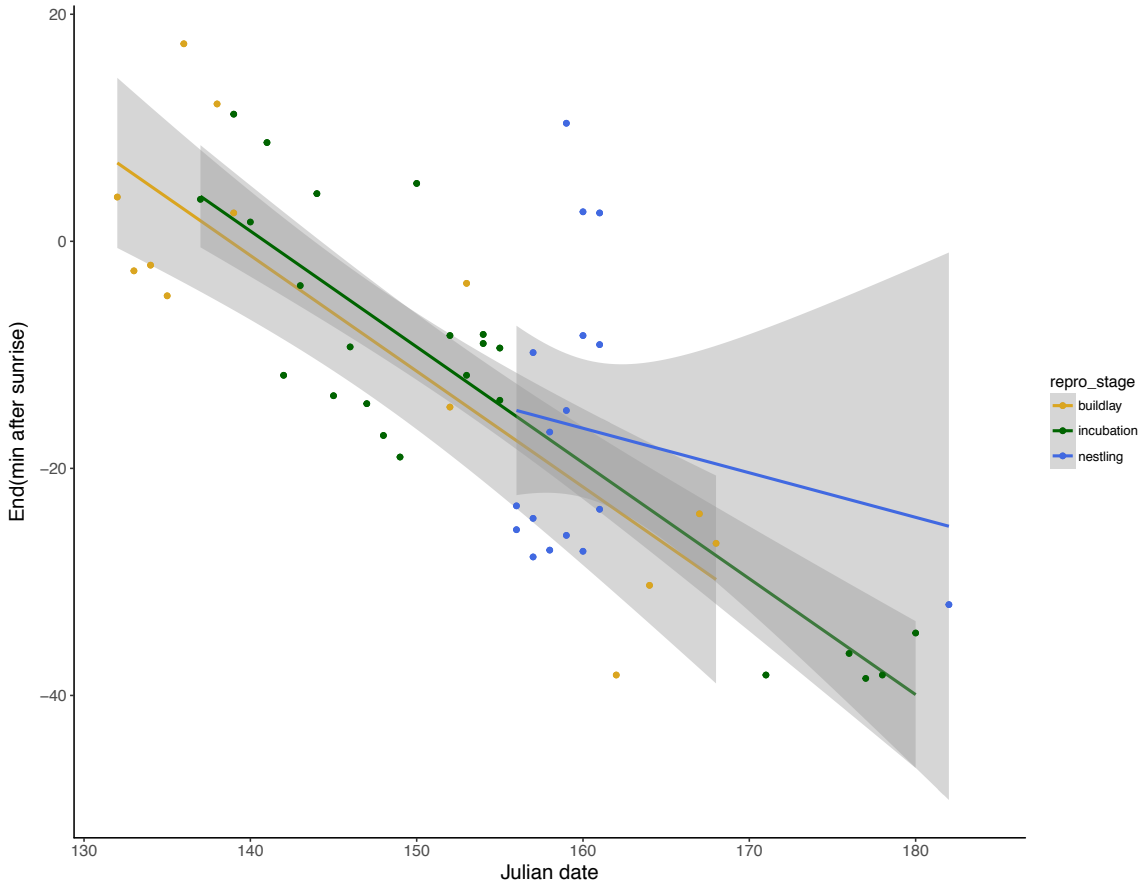

Figure 11. Model-averaged prediction for the influence of breeding stage (build/lay, incubation, nestling) on the end time of the dawn song bout in relation to sunrise of paired male Cerulean Warblers in 2017 in south-central Indiana. Early in the season (May), regardless of breeding stage, males ended dawn song bouts after sunrise; however, dawn song bouts decreased steadily as the season progressed. Shaded gray areas represent 95% confidence intervals.

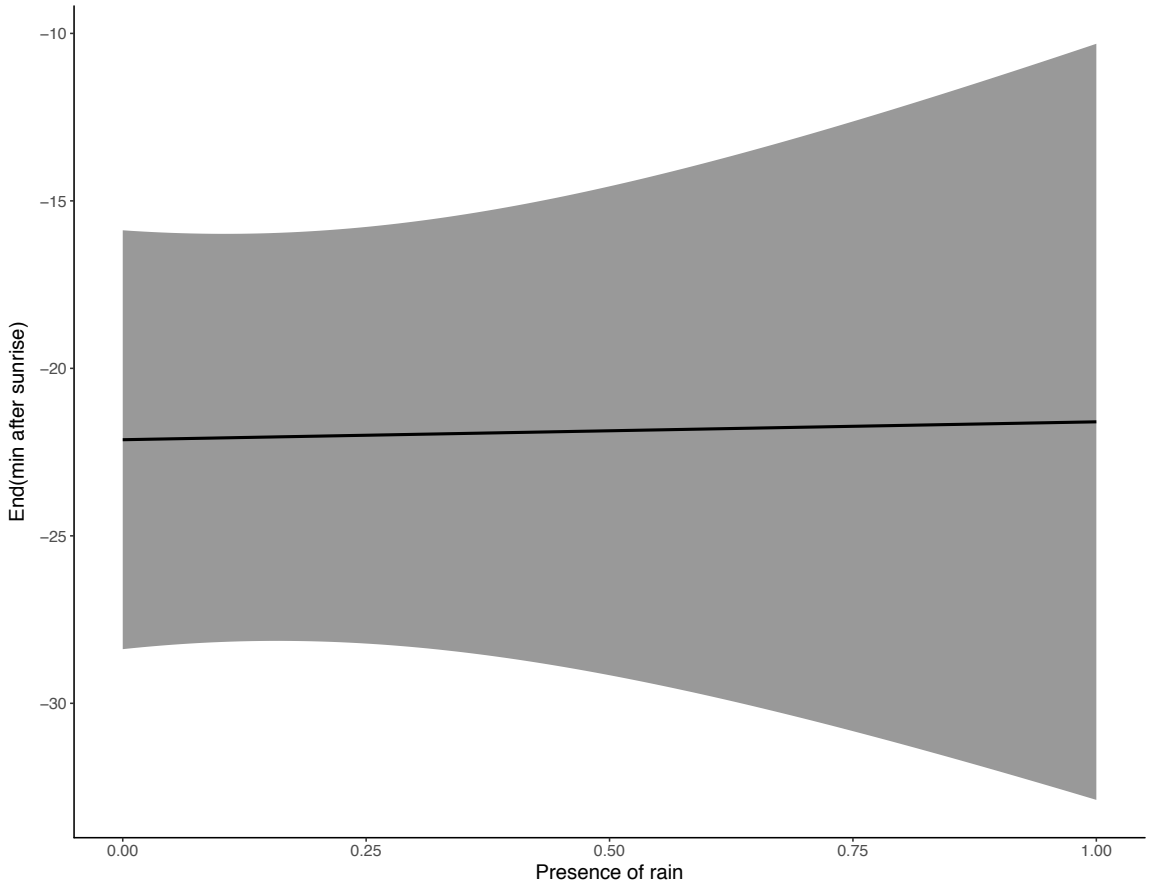

Figure 12. Model-averaged prediction for the influence of the presence of rain on the end time of the dawn song bout in relation to sunrise of paired male Cerulean Warblers in 2017 in south-central Indiana. Males ended dawn song bouts slightly later on mornings with rain. Shaded gray areas represent 95% confidence intervals.

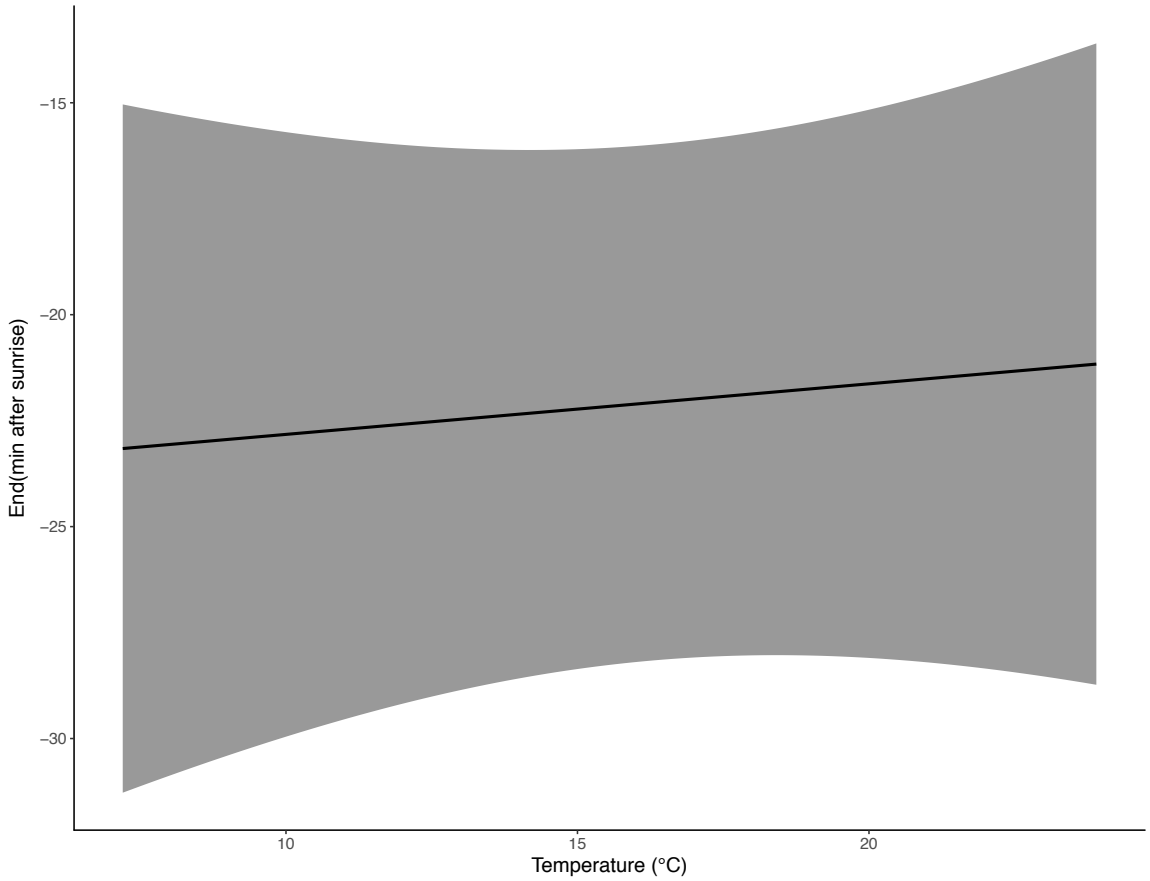

Figure 13. Model-averaged prediction for the influence of temperature on the end time of the dawn song bout in relation to sunrise of paired male Cerulean Warblers in 2017 in south-central Indiana. Males ended dawn song bouts later on mornings that were warm. Shaded gray areas represent 95% confidence intervals.

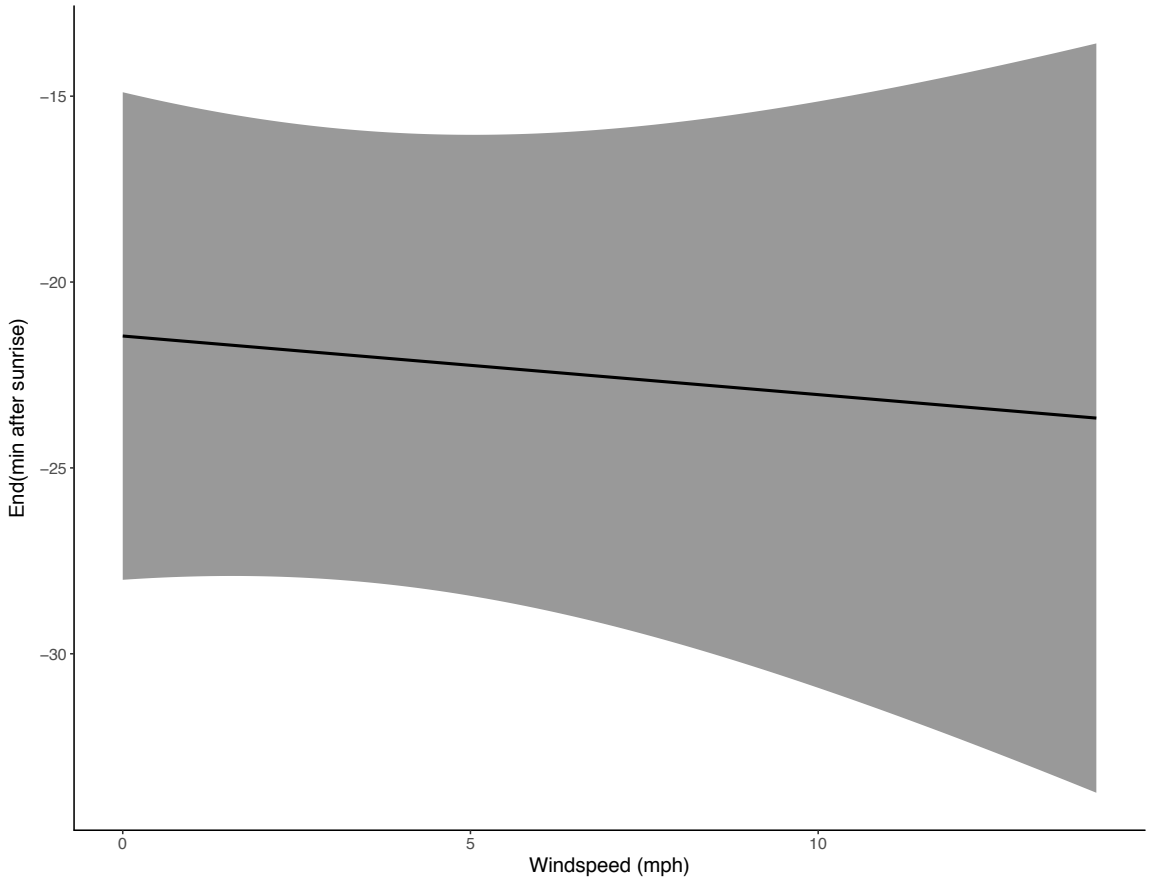

Figure 14. Model-averaged prediction for the influence of wind on the end time of the dawn song bout in relation to sunrise of paired male Cerulean Warblers in 2017 in south-central Indiana. Males ended dawn song bouts earlier on windy mornings. Shaded gray areas represent 95% confidence intervals.

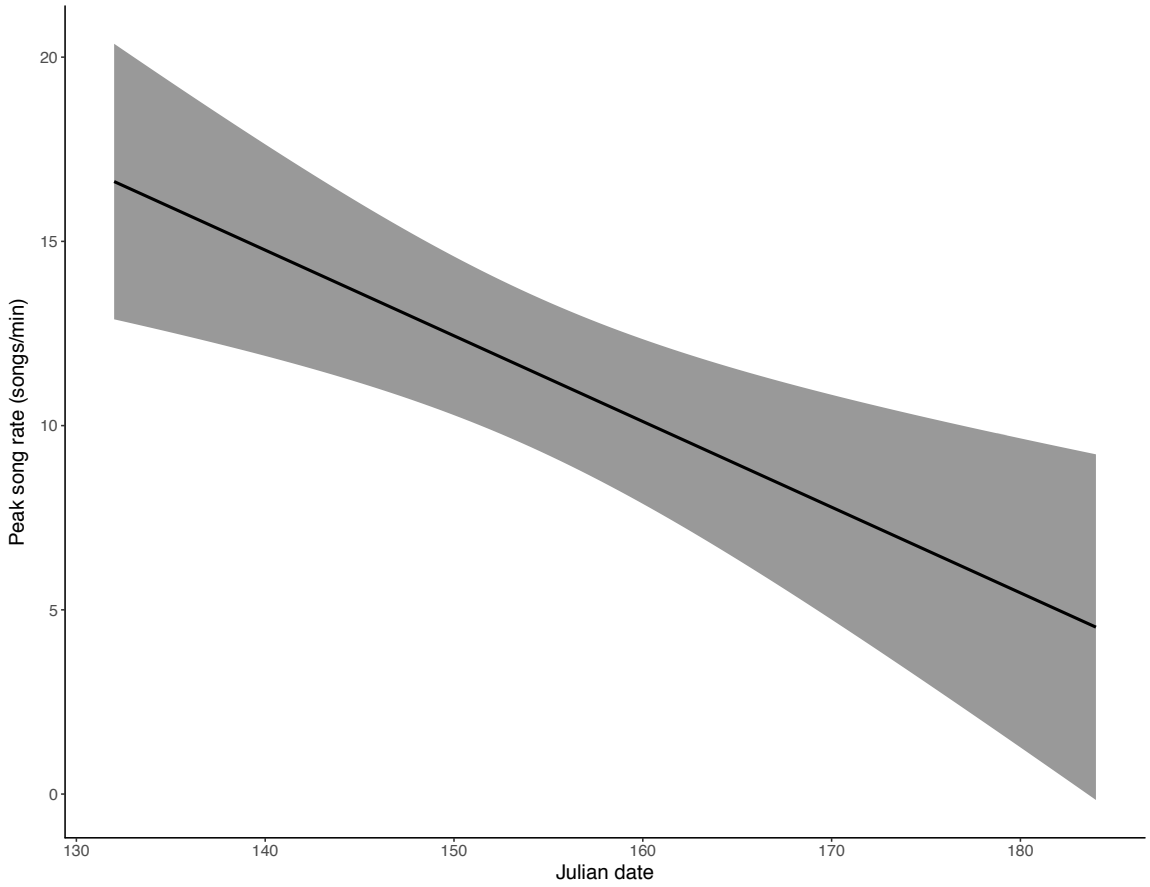

Figure 15. Model-averaged prediction for the influence of Julian date on the peak song rate of the dawn song bout of paired male Cerulean Warblers in 2017 in south-central Indiana. Peak dawn song rate declined steeply as the breeding season progressed. Shaded gray areas represent 95% confidence intervals.

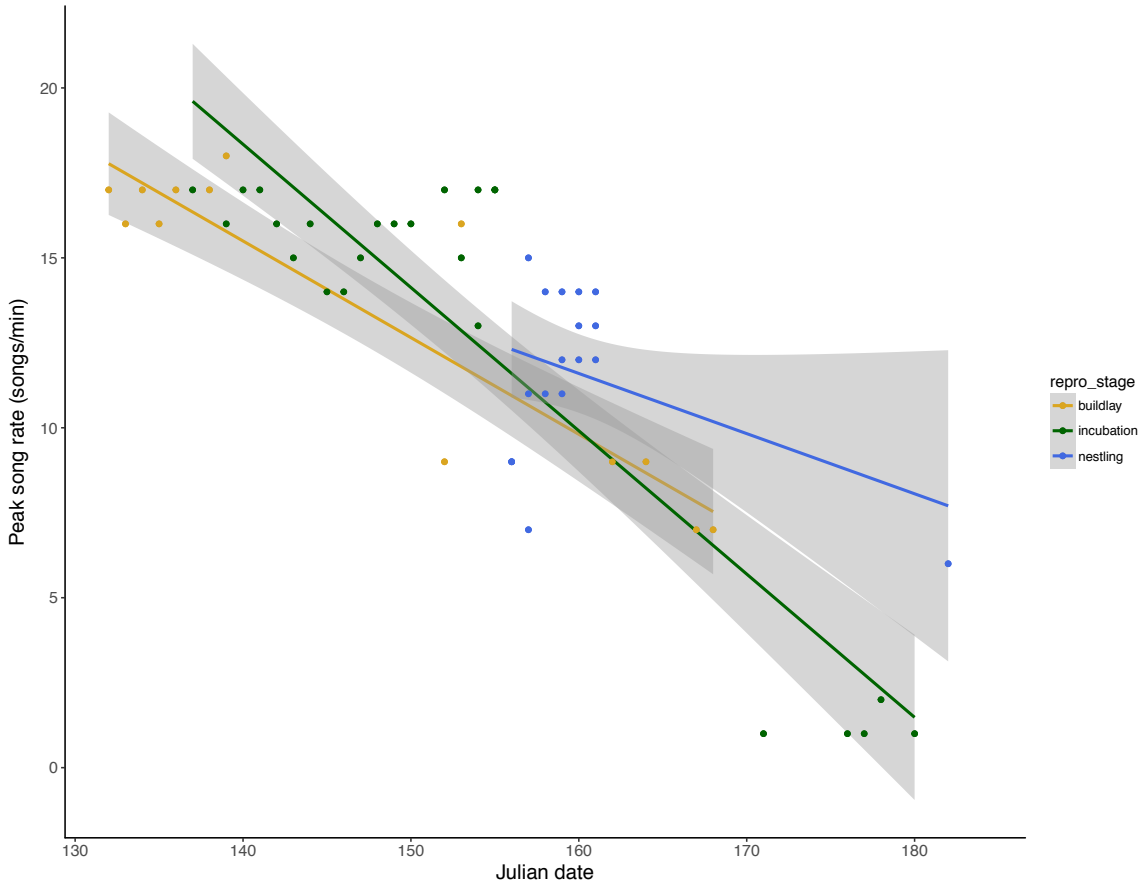

Figure 16. Model-averaged prediction for the influence of breeding stage (build/lay, incubation, nestling) on the peak song rate of the dawn song bout of paired male Cerulean Warblers in 2017 in south-central Indiana. Peak dawn song rate decreased steadily as the season progressed, regardless of breeding stage. Shaded gray areas represent 95% confidence intervals.

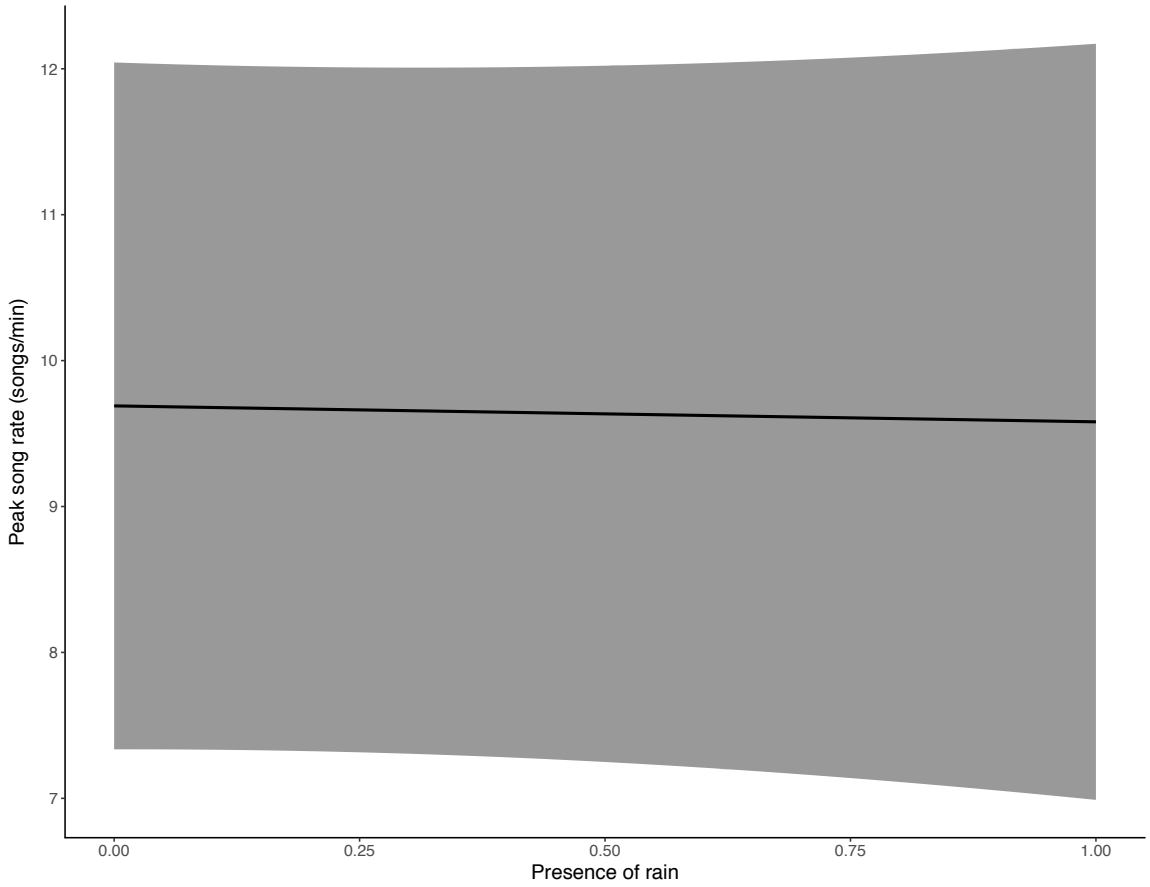

Figure 17. Model-averaged prediction for the influence of the presence of rain on the peak song rate of the dawn song bout of paired male Cerulean Warblers in 2017 in south-central Indiana. Peak song rate was slightly negatively correlated with the presence of rain. Shaded gray areas represent 95% confidence intervals.

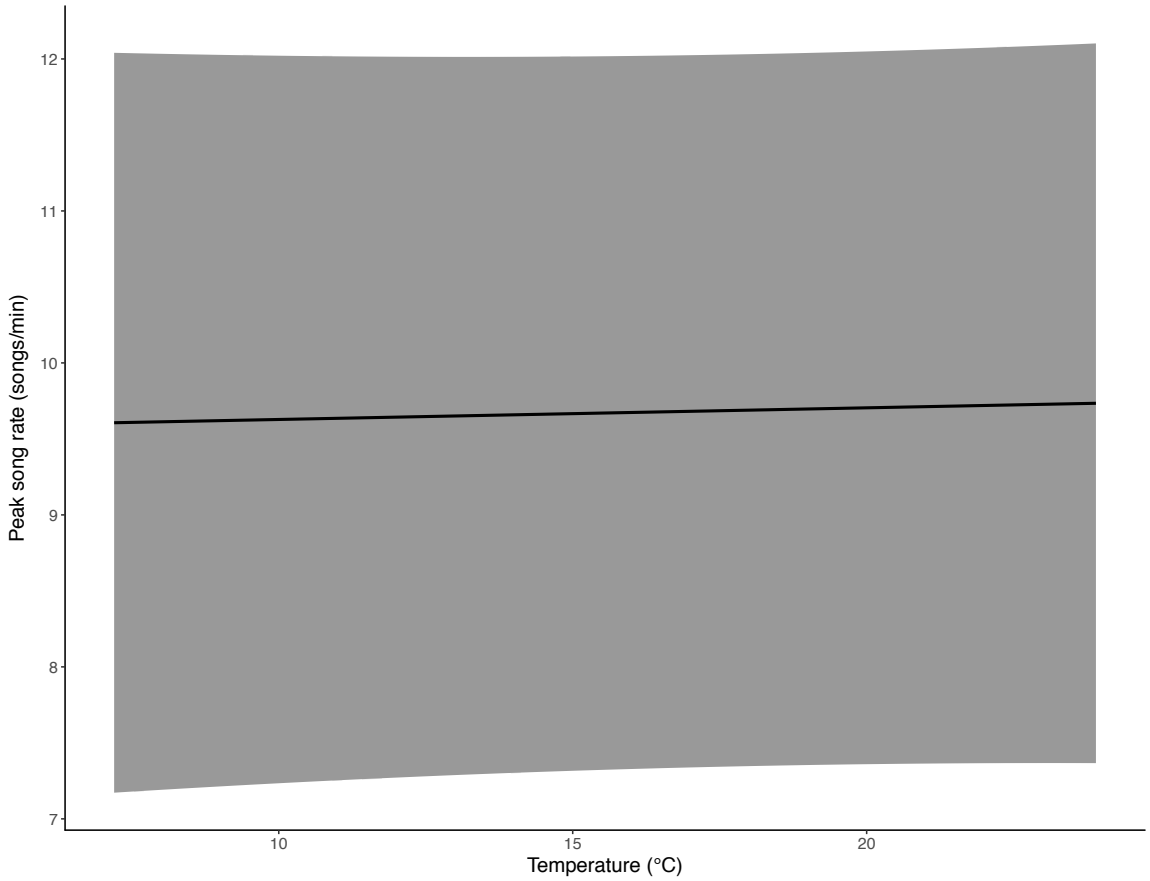

Figure 18. Model-averaged prediction for the influence of temperature on the peak song rate of the dawn song bout of paired male Cerulean Warblers in 2017 in south-central Indiana. Peak song rate was positively correlated with temperature. Shaded gray areas represent 95% confidence intervals.

CHAPTER 2 TABLES

Table 1. Response variables used to characterize the dawn singing behavior of paired male Cerulean Warblers in south-central Indiana in 2017.

Variable Name	Description
YES/NO	If dawn song occurred or not
BOUT LENGTH	Length of the dawn song bout (min)
START	Number of minutes before sunrise of first song
END	Number of minutes in relation to sunrise of last song
AVERAGE	Average of per min song rates during dawn song bout
PEAK	Peak per min song rate during dawn song bout

Table 2. Predictor variables used to characterize the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017.

Variable Name	Description
JULIAN DATE	The elapsed time in days since 1 January
BREEDING STAGE	
BUILD/LAY	Combination of breeding stages lasting ~ 6 days (build) and ~ 4 days (lay)
INCUBATION	Breeding stage lasting ~ 12 days
NESTLING	Breeding stage lasting ~ 11 days
WEATHER	
TEMPERATURE	Air temperature given in whole degrees Celsius (°C)
WIND	Speed of the wind given in miles per hour (mph)
RAIN	Binary: yes/no regarding the occurrence of rain during the dawn song bout

Table 3. Summary of model selection results for the presence of the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017 as a function of seasonality, breeding stage, and weather. Models are ranked by ascending value of Akaike's Information Criterion corrected for small sample sizes (AICc); w_i is the model weight, and df is the number of parameters. All models also included a random effect ("individual"). Models with ΔAICc values of < 2.0 were considered equally plausible.

Model	ΔAICc	df	w_i
Julian date	0.0	3	0.2655
Julian date + temperature	0.9	4	0.1658
Julian date + wind	1.2	4	0.1458
Julian date + rain	2.0	4	0.0970
Julian date + temperature + wind	3.0	5	0.0589
Julian date + temperature + rain	3.1	5	0.0550
Julian date + wind + rain	3.4	5	0.0482
Julian date + breeding stage	4.3	5	0.0306
Julian date + temperature + wind + rain	5.3	6	0.0190
Julian date + breeding stage + temperature	5.3	6	0.0184
Julian date + breeding stage + wind	5.5	6	0.0166
Julian date + breeding stage + rain	6.4	6	0.0109
Null model	6.5	2	0.0102
Julian date + breeding stage + temperature + wind	7.5	7	0.0063
Julian date + breeding stage + temperature + rain	7.6	7	0.0058
Wind	7.7	3	0.0055
Breeding stage	7.7	4	0.0055
Julian date + breeding stage + wind + rain	7.9	7	0.0052
Temperature	8.0	3	0.0049
Rain	8.6	3	0.0036
Breeding stage + temperature	8.6	5	0.0036
Breeding stage + wind	8.6	5	0.0035
Temperature + wind	9.8	4	0.0019
Julian date + breeding stage + temperature + wind + rain	9.9	8	0.0019
Wind + rain	9.9	4	0.0019
Breeding stage + rain	9.9	5	0.0019
Temperature + rain	10.2	4	0.0017
Breeding stage + temperature + wind	10.6	6	0.0013
Breeding stage + wind + rain	10.8	6	0.0012
Breeding stage + temperature + rain	10.9	6	0.0012
Temperature + wind + rain	12.0	5	<0.001
Breeding stage + temperature + wind + rain	12.8	7	<0.001

Table 4. Model-averaged estimates of factors affecting the presence of the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017.

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)	Relative Variable Importance	N containing models
Intercept	0.88152	1.14875	1.16335	0.758	0.44861	-	-
Julian date	-2.85286	1.06757	1.08112	2.639	0.00832	1.00	3
Temperature	-0.13594	0.31548	0.31766	0.428	0.66869	0.25	1
Wind	-0.09502	0.25471	0.25663	0.370	0.71119	0.22	1

Table 5. Summary of model selection results for dawn song bout length of paired male Cerulean Warblers in south-central Indiana in 2017 as a function of seasonality, breeding stage, and weather. Models are ranked by ascending value of Akaike's Information Criterion corrected for small sample sizes (AICc); w_i is the model weight, and df is the number of parameters. All models also included a random effect ("individual"). Models with ΔAICc values of < 2.0 were considered equally plausible.

Model	ΔAICc	df	w_i
Julian date + breeding stage + rain	0.0	7	0.2260
Julian date + breeding stage	0.7	6	0.1628
Julian date + breeding stage + temperature + rain	2.3	8	0.0700
Julian date + breeding stage + wind + rain	2.4	8	0.0693
Julian date + breeding stage + wind	2.4	7	0.0668
Julian date + breeding stage + temperature	2.9	7	0.0543
Julian date + temperature + rain	3.5	6	0.0384
Julian date	3.6	4	0.0365
Julian date + wind + rain	3.7	6	0.0358
Julian date + breeding stage + temperature + wind + rain	4.8	9	0.0209
Julian date + breeding stage + temperature + wind	4.8	8	0.0205
Julian date + temperature	5.4	5	0.0155
Julian date + wind	5.6	5	0.0138
Julian date + temperature + wind + rain	5.6	7	0.0134
Null model	6.7	3	0.0078
Rain	6.9	4	0.0073
Breeding stage + rain	7.4	6	0.0056
Julian date + temperature + wind	7.6	6	0.0051
Temperature	8.8	4	0.0027
Wind	8.9	4	0.0027
Wind + rain	9.0	5	0.0025
Temperature + rain	9.1	5	0.0024
Breeding stage + wind + rain	9.2	7	0.0023
Breeding stage	9.2	5	0.0023
Breeding stage + temperature + rain	9.7	7	0.0018
Temperature + wind	11.0	5	<0.001
Temperature + wind + rain	11.2	6	<0.001
Breeding stage + temperature	11.4	6	<0.001
Breeding stage + temperature + wind + rain	11.4	8	<0.001
Breeding stage + wind	11.4	6	<0.001
Breeding stage + temperature + wind	13.7	7	<0.001
Julian date + rain	NA	NA	NA

Table 6. Model-averaged estimates of factors affecting the length of the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017.

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)	Relative Variable Importance	N containing models
Intercept	1.85107	0.40304	0.41085	4.505	6.60e-06	-	-
Julian date	-1.16652	0.36348	0.36794	3.17	0.00152	1.00	3
Breeding stage	-	-	-	-	-	0.74	2
Incubation	-0.06215	0.26437	0.2711	0.229	0.81867	-	-
Nestling	0.78234	0.63526	0.64275	1.217	0.22353	-	-
Rain	-0.52148	0.45359	0.45718	1.141	0.25401	0.66	2

Table 7. Summary of model selection results for the starting time of the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017 as a function of seasonality, breeding stage, and weather. Models are ranked by ascending value of Akaike's Information Criterion corrected for small sample sizes (AICc); w_i is the model weight, and df is the number of parameters. All models also included a random effect ("individual"). Models with ΔAICc values of < 2.0 were considered equally plausible.

Model	ΔAICc	df	w_i
Wind + rain	0.0	5	0.2318
Temperature + rain	1.3	5	0.1232
Rain	1.5	4	0.1120
Temperature + wind + rain	1.8	6	0.0920
Julian date + wind + rain	2.1	6	0.0801
Wind	2.5	4	0.0669
Julian date + temperature + rain	3.4	6	0.0434
Julian date + rain	3.5	5	0.0401
Julian date + temperature + wind + rain	4.0	7	0.0312
Breeding stage + wind + rain	4.2	7	0.0281
Temperature + wind	4.4	5	0.0254
Julian date + wind	4.6	5	0.0234
Breeding stage + temperature + rain	5.3	7	0.0168
Breeding stage + rain	5.5	6	0.0146
Breeding stage + temperature + wind + rain	6.1	8	0.0110
Julian date + breeding stage + wind + rain	6.3	8	0.0098
Julian date + temperature + wind	6.6	6	0.0087
Breeding stage + wind	6.7	6	0.0081
Julian date + breeding stage + temperature + rain	7.0	8	0.0070
Julian date + breeding stage + rain	7.4	7	0.0059
Temperature	7.6	4	0.0052
Julian date + breeding stage + temperature + wind + rain	8.1	9	0.0040
Breeding stage + temperature + wind	8.8	7	0.0029
Julian date + breeding stage + wind	9.0	7	0.0026
Julian date + temperature	9.6	5	0.0019
Null model	10.1	3	0.0015
Julian date + breeding stage + temperature + wind	11.1	8	<0.001
Breeding stage + temperature	12.0	6	<0.001
Julian date	12.1	4	<0.001
Julian date + breeding stage + temperature	14.1	7	<0.001
Breeding stage	14.4	5	<0.001
Julian date + breeding stage	16.6	6	<0.001

Table 8. Model-averaged estimates of factors affecting the starting time in relation to sunrise of the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017.

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)	Relative Variable Importance	N containing models
Intercept	3.626734	0.026082	0.026725	135.704	<2e-16	-	-
Wind	-0.015846	0.018731	0.018933	0.837	0.4026	1.00	4
Rain	-0.144692	0.058090	0.059349	2.438	0.0148	0.56	2
Temperature	-0.006748	0.013583	0.013757	0.490	0.6238	0.36	2

Table 9. Summary of model selection results for the ending time of the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017 as a function of seasonality, breeding stage, and weather. Models are ranked by ascending value of Akaike's Information Criterion corrected for small sample sizes (AICc); w_i is the model weight, and df is the number of parameters. All models also included a random effect ("individual"). Models with ΔAICc values of < 2.0 were considered equally plausible.

Model	ΔAICc	df	w_i
Julian date + breeding stage + temperature + wind + rain	0.0	9	0.2889
Julian date + breeding stage + wind + rain	1.0	8	0.1791
Julian date + breeding stage + temperature + rain	1.1	8	0.1700
Julian date + breeding stage + rain	1.4	7	0.1427
Julian date + breeding stage + temperature + wind	3.0	8	0.0645
Julian date + breeding stage + temperature	3.8	7	0.0433
Julian date + breeding stage + wind	4.0	7	0.0398
Julian date + breeding stage	4.1	6	0.0369
Julian date + temperature + wind + rain	6.8	7	0.0094
Julian date + wind + rain	7.6	6	0.0066
Julian date + temperature + rain	7.6	6	0.0065
Julian date + rain	8.0	5	0.0053
Julian date + temperature + wind	9.9	6	0.0021
Julian date + temperature	10.6	5	0.0014
Julian date + wind	10.7	5	0.0014
Julian date	10.9	4	0.0012
Breeding stage + temperature + wind + rain	14.2	8	<0.001
Breeding stage + temperature + rain	15.0	7	<0.001
Breeding stage + wind + rain	15.3	7	<0.001
Breeding stage + rain	15.8	6	<0.001
Breeding stage + temperature + wind	17.4	7	<0.001
Breeding stage + temperature	18.0	6	<0.001
Breeding stage + wind	18.6	6	<0.001
Breeding stage	18.7	5	<0.001
Temperature + wind + rain	21.2	6	<0.001
Temperature + rain	22.2	5	<0.001
Wind + rain	22.6	5	<0.001
Rain	23.1	4	<0.001
Temperature + wind	24.3	5	<0.001
Temperature	25.0	4	<0.001
Wind	25.7	4	<0.001
Null model	26.0	3	<0.001

Table 10. Model-averaged estimates of factors affecting the ending time in relation to sunrise of the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017.

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)	Relative Variable Importance	N containing models
Intercept	-23.0525	4.0962	4.2066	5.480	<2e-16	-	-
Julian date	-13.2737	2.4876	2.5547	5.196	2.00e-07	1.00	4
Breeding stage	-	-	-	-	-	1.00	4
Incubation	0.9408	3.6659	3.7650	0.250	0.803	-	-
Nestling	7.0177	5.1742	5.3136	1.321	0.187	-	-
Temperature	0.4665	1.2855	1.3156	0.355	0.723	0.53	2
Wind	-0.5117	1.3541	1.3857	0.369	0.712	0.54	2
Rain	0.4415	5.5358	5.6829	0.078	0.938	1.00	4

Table 11. Summary of model selection results for the average song rate during the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017 as a function of seasonality, breeding stage, and weather. Models are ranked by ascending value of Akaike's Information Criterion corrected for small sample sizes (AICc); w_i is the model weight, and df is the number of parameters. All models also included a random effect ("individual"). Models with ΔAICc values of < 2.0 were considered equally plausible.

Model	ΔAICc	df	w_i
Julian date + breeding stage	0.0	6	0.1261
Julian date + breeding stage + rain	0.1	7	0.1191
Julian date + rain	0.7	5	0.0882
Julian date	1.0	4	0.0770
Null model	1.6	3	0.0557
Rain	1.7	4	0.0530
Julian date + breeding stage + wind	2.4	7	0.0380
Julian date + breeding stage + wind + rain	2.5	8	0.0359
Breeding stage + rain	2.6	6	0.0339
Julian date + breeding stage + temperature + rain	2.7	8	0.0335
Julian date + breeding stage + temperature	2.7	7	0.0326
Breeding stage	2.8	5	0.0308
Julian date + temperature + rain	3.0	6	0.0281
Julian date + wind + rain	3.5	6	0.0224
Julian date + temperature	3.6	5	0.0210
Julian date + wind	3.6	5	0.0206
Temperature + rain	3.7	5	0.0200
Julian date + breeding stage + temperature + wind	3.8	8	0.0191
Temperature	3.9	4	0.0176
Julian date + breeding stage + temperature + wind + rain	4.0	9	0.0174
Wind	4.4	4	0.0138
Wind + rain	4.5	5	0.0133
Breeding stage + temperature + rain	4.7	7	0.0118
Julian date + temperature + wind + rain	4.9	7	0.0107
Julian date + temperature + wind	5.0	6	0.0105
Temperature + wind	5.3	5	0.0090
Breeding stage + temperature	5.3	6	0.0088
Temperature + wind + rain	5.4	6	0.0083
Breeding stage + wind + rain	5.5	7	0.0081
Breeding stage + wind	5.7	6	0.0074
Breeding stage + temperature + wind + rain	6.8	8	0.0042
Breeding stage + temperature + wind	6.8	7	0.0041

Table 12. Summary of model selection results for the peak song rate during the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017 as a function of seasonality, breeding stage, and weather. Models are ranked by ascending value of Akaike's Information Criterion corrected for small sample sizes (AICc); w_i is the model weight, and df is the number of parameters. All models also included a random effect ("individual"). Models with ΔAICc values of < 2.0 were considered equally plausible.

Model	ΔAICc	df	w_i
Julian date + breeding stage	0.0	6	0.1992
Julian date + breeding stage + rain	0.4	7	0.1660
Julian date	1.5	4	0.0930
Julian date + rain	1.7	5	0.0835
Julian date + breeding stage + temperature	2.0	7	0.0740
Julian date + breeding stage + temperature + rain	2.2	8	0.0663
Julian date + breeding stage + wind	3.1	7	0.0432
Julian date + breeding stage + wind + rain	3.1	8	0.0413
Julian date + temperature + rain	3.4	6	0.0360
Julian date + temperature	3.5	5	0.0347
Julian date + breeding stage + temperature + wind	4.2	8	0.0247
Julian date + breeding stage + temperature + wind + rain	4.3	9	0.0227
Julian date + wind + rain	4.4	6	0.0220
Julian date + wind	4.5	5	0.0205
Breeding stage	5.5	5	0.0126
Breeding stage + rain	5.6	6	0.0119
Julian date + temperature + wind + rain	5.9	7	0.0105
Julian date + temperature + wind	5.9	6	0.0104
Breeding stage + temperature + rain	6.5	7	0.0077
Breeding stage + temperature	6.9	6	0.0063
Breeding stage + wind + rain	7.9	7	0.0039
Breeding stage + wind	8.5	6	0.0028
Breeding stage + temperature + wind + rain	9.1	8	0.0021
Breeding stage + temperature + wind	9.6	7	0.0017
Null model	11.0	3	<0.001
Rain	11.3	4	<0.001
Temperature	12.0	4	<0.001
Temperature + rain	12.3	5	<0.001
Wind	13.8	4	<0.001
Wind + rain	13.8	5	<0.001
Temperature + wind	14.4	5	<0.001
Temperature + wind + rain	14.5	6	<0.001

Table 13. Model-averaged estimates of factors affecting peak song rate during the dawn song bout of paired male Cerulean Warblers in south-central Indiana in 2017.

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)	Relative Variable Importance	N containing models
Intercept	9.75998	1.3358	1.36617	7.144	<2e-16	-	-
Julian date	-3.18159	1.0133	1.02966	3.090	2.00e-03	1.00	5
Breeding stage	-	-	-	-	-	0.65	3
Incubation	-0.01076	0.6672	0.68458	0.016	0.987	-	-
Nestling	1.32096	1.5568	1.58199	0.835	0.404	-	-
Rain	-0.11059	0.6538	0.66980	0.165	0.869	0.38	2
Temperature	0.02923	0.1286	0.13042	0.224	0.823	0.10	1

CHAPTER 3: American Redstarts (*Setophaga ruticilla*) usurp Cerulean Warbler (*Setophaga cerulea*) nest in Southern Indiana

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1. ABSTRACT

Documented observations of interspecific nest usurpation are rare and have been observed mostly in cavity-nesting species, where cavities are limiting and competition for them can be strong. We report one of the few known instances of interspecific nest usurpation in the family Parulidae (North American wood-warblers) between the Cerulean Warbler (*Setophaga cerulea*) and American Redstart (*Setophaga ruticilla*). During May and June 2016, we observed a female American Redstart usurp and successfully fledge at least one of its young from a nest built by a female Cerulean Warbler in Yellowwood State Forest, Brown County, Indiana. Based on our field observations, we provide information on nest chronology and describe American Redstart adult behaviors at the usurped nest. We hypothesize that this instance of interspecific nest usurpation may have been a result of high local breeding densities of both Cerulean Warblers and American Redstarts in our study area.

Keywords: American Redstart, Cerulean Warbler, interspecific competition, nest usurpation, Parulidae, *Setophaga*

2. INTRODUCTION

Interspecific nest usurpation is a rarely documented phenomenon in which 2 species converge on a nest niche, generally resulting in a winner and a loser (Lindell 1996). Usually, one individual gains access to the nest, and the individual that built the nest is evicted, although interspecific nest sharing has been observed (Robinson et al. 2005). Most documented cases of nest usurpation involve cavity-nesting species, whereby specific habitat requirements limit the number of potential nesting sites, often leading to intense competition (Lindell 1996). Families in which nest usurpation of cavities has been documented include Picidae (Red-bellied Woodpecker [*Melanerpes carolinus*]; Shackelford et al. 2000), Sturnidae (European Starling [*Sturnus vulgaris*]; Lindell 1996), Troglodytidae (House Wren [*Troglodytes aedon*]; Johnson 2014), Turdidae (Eastern Bluebird [*Sialia sialia*]; Gowaty and Plissner 2015), and Passeridae (House Sparrow [*Passer domesticus*]; Tarof and Brown 2013).

Nest usurpation in open-cup nesting birds is much rarer than in cavity-nesting species (Lindell 1996) but has been reported in several avian families, including Corvidae (Mexican Jay [*Aphelocoma wollweberi*]; McCormack and Brown 2008) and Columbidae (Eurasian Collared-Dove [*Streptopelia decaocto*]; Kasner and Pyeatt 2016). Studying the circumstances in which this behavior occurs can lead to an improved understanding of the evolution of nest site selection in different species. Here we report the first instance of interspecific usurpation of a Cerulean Warbler nest followed by successful fledging of young by an American Redstart pair.

3. METHODS

Nest observations occurred in Yellowwood State Forest (39°06′39.8484″N, 86°19′30.133″W), Brown County, Indiana, as part of the Hardwood Ecosystem Experiment, a long-term (100-year), multi-taxa, multi-organizational study initiated in 2006 (Swihart et al.

2013). The study area consists of deciduous hardwood forest with >50% of the canopy dominated by oaks (*Quercus* spp.) and hickories (*Carya* spp.) >80 years old (Swihart et al. 2013) with a mid-story of maple (*Acer* spp.) and tulip-poplar (*Liriodendron tulipifera*). The area around the usurped nest had an understory dominated by multiflora rose (*Rosa multiflora*) and ironwood (*Ostrya virginiana*) with an average canopy height of 22 m. We observed the nest with binoculars and spotting scopes for 30 min every 1–3 d from 20 May to 8 June 2016 (intervals between observations decreased as nesting progressed). Nest observations were conducted opportunistically during daylight hours.

4. RESULTS

We located a Cerulean Warbler nest on 20 May 2016 in Yellowwood State Forest by hearing a female produce a *zeet* call to her singing mate while she was on the nest. The nest was adjacent to an infrequently used forest road, 9.0 m high in a 12.4 m white oak (*Quercus alba*). During our first nest observation on the discovery date, we surmised that the nest was in the incubation stage; the female was on the nest for most of the observation period, and the nest appeared fully constructed. Female Cerulean Warblers rarely visit their nests after the completion of nest building and before incubation begins; once incubation commences, female Cerulean Warblers will sit on their nests for long periods of time (CDD and GJM, pers. obs.). During the next observation on 22 May, a male American Redstart landed briefly on the nest branch while the female Cerulean Warbler was off the nest. The female Cerulean Warbler still appeared to be incubating, as she did not stay off the nest for long (average of 2.5 min for 3 trips). On 24 May, we found a female American Redstart sitting on the Cerulean Warbler nest. A male American Redstart sang nearby (<10 m from nest), while the male Cerulean Warbler *whisper* sang (singing softly) close-by. Both the male and female American Redstarts were very

vocal; the female American Redstart often chipped from the nest in response to her mate. Near the end of the observation, the female Cerulean Warbler sat on the nest for a short period of time (<1 min) until the American Redstart female returned to the nest after being absent for 7 min. Nesting stage was recorded as incubation because the female American Redstart sat on the nest for the majority of the observation period. We were unable to see the nest's contents because of its height and concealment from nearby vegetation. We looked for eggs and eggshells below the nest that may have belonged to the Cerulean Warbler pair, but none was found; however, based on the location of the nest and the behavior of the female Cerulean Warbler, we determined that the nest was constructed and initially occupied by the female Cerulean Warbler.

On 26 May, we observed the female American Redstart sitting on the nest; however, because of a storm with heavy rain, our observation time was reduced. The next day (27 May), the female American Redstart was on the nest for 26 min during the 30 min observation period. Two days later (29 May), the female American Redstart remained on the nest during the entire observation period while her mate sang within 30 m of the nest. There was no sign of the female Cerulean Warbler, but her presumed mate sang about 40 m away. On 31 May, the female American Redstart arrived to the nest and poked her head inside before sitting to incubate. She occasionally stood on the nest and seemed to look at the nest contents before repositioning herself. Her mate was observed singing within 20 m of the nest and he briefly sang from the nest tree while the female American Redstart was off the nest. The male Cerulean Warbler was heard singing within 30 m of the nest during this observation. On 2 June, the female American Redstart again poked her head into the nest, seemed to examine the nest contents, and shifted her body position on the nest at short intervals; we surmised that the eggs were close to hatching. On 4 June, we saw the female Cerulean Warbler return to the nest, look inside, and leave within a few

seconds. Shortly after, the female American Redstart returned to sit on the nest. A few minutes later, her mate returned carrying food to nestlings, and then both parents continued to feed the nestlings for the rest of the observation period. On 7 June, we determined there were two nestlings based on bills visible from the nest rim. Both parents fed nestlings throughout the observation period and occasionally removed fecal sacs. One nestling fledged on 8 June. We found it on the ground below the nest being cared for by both adults; the other nestling was never seen out of the nest. We visually determined that the fledgling most closely resembled an American Redstart because American Redstart and Cerulean Warbler fledglings are markedly different from one another in plumage coloration (Sherry and Holmes 1997, Baicich and Harrison 2005).

5. DISCUSSION

To our knowledge, this is the first documentation of interspecific nest usurpation followed by successful fledging of young between 2 species of parulids (wood-warblers). Aggressive interactions between Cerulean Warblers and American Redstarts have been documented in the past, and American Redstarts have been noted as the aggressor in most situations involving interspecific interactions with Cerulean Warblers (Boves and Buehler 2012, Buehler et al. 2013). Why 2 previous accounts of nest usurpations between Cerulean Warblers and American Redstarts have not successfully fledged young is unknown (Boves and Buehler 2012). Published accounts of nesting locations typically differ between the 2 species: Cerulean Warbler nests are usually placed on a horizontal limb of a deciduous tree in the mid-story or over-story canopy (Rogers 2006, Roth and Islam 2008, Buehler et al. 2013, Wagner and Islam 2014), whereas American Redstart nests are generally placed in trees with a smaller diameter-at-breast-height (DBH) and are normally located against the main stem of the tree (Sherry and

Holmes 1997). In southern Indiana, white oak (*Quercus alba*) is the preferred nest tree of Cerulean Warblers (Roth and Islam 2008, Wagner and Islam 2014), whereas anecdotal observations by the authors suggest the preference of American beech (*Fagus grandifolia*) as nest trees by American Redstarts.

In the same study unit where the usurpation of the Cerulean Warbler nest occurred, we found Cerulean Warblers and American Redstarts in higher densities than in our other study units (CDD and GJM, unpubl. data). Thus, the chance for interspecific competition to occur would potentially be higher between the two species at this study unit.

American Redstarts and Cerulean Warblers arrive on the breeding grounds in Indiana at approximately the same time (mid-Apr to early-May; eBird 2012), and nesting commences shortly thereafter (CDD and GJM, pers. obs.). Because this incident occurred at the end of May, the American Redstart pair could have had a first nesting attempt that failed. This could have promoted the usurpation of an already constructed nest as a time and energy saving strategy. The habitat preferences of Cerulean Warblers seem to be more specific, whereas American Redstarts seem to be more of a habitat generalist (pers. obs.). At our study area, American Redstarts are found in most study units, whereas Cerulean Warblers only occur in specific locations. In the study unit where we observed nest usurpation, Cerulean Warblers typically occurred on southeast facing slopes or in bottomlands where oaks and hickories were common. In contrast, American Redstarts inhabited additional areas where Cerulean Warblers were not located, such as areas dominated by American beech or maple (*Acer* spp.). Where these two species co-occur, we have anecdotally observed nests close to each other. For example, we observed Cerulean Warbler and American Redstart nests within 15 m of each other in the same study unit where the nest usurpation occurred. From 2010 to 2015, Cerulean Warbler nest heights at our study units

ranged from 9.0 to 29.5 m (n = 106). Nest height at the usurped nest was much lower than the average Cerulean Warbler nest height of 18 m in our study population (Wagner and Islam 2014). The female Cerulean Warbler constructed a nest at a height of 9.0 m, which is close to the American Redstart nest height average of 8.2 m (Hubbard Brook Experimental Forest, New Hampshire, USA; Sherry and Holmes 1997), thus providing an opportunity for the more aggressive American Redstart to usurp an already constructed nest.

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CHAPTER 4: Female song documented in two Cerulean Warblers (*Setophaga cerulea*)

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1. ABSTRACT

Female song is now known to be widespread and ancestral, yet it is still poorly understood, particularly in temperate regions. We document the singing behavior of 2 mated female Cerulean Warblers (*Setophaga cerulea*) in southern Indiana in June 2017; this is the first documentation of female song in this species. When assessed aurally and spectrographically, the songs of both females resembled neither each other, nor any previously documented Cerulean Warbler vocalization. Female song in this species may function intersexually as a means of reinforcing the male-female pair bond when resource competition is high, but more data are needed to test this hypothesis.

Keywords: Cerulean Warbler, female song, Parulidae, *Setophaga cerulea*, wood-warbler

2. INTRODUCTION

Female song is more prevalent than once thought and is now believed to be ancestral (Odom et al. 2014). In tropical and sub-tropical regions, females of many species are prolific singers: both solo female song and song duets by male-female pairs are common (e.g., Cooney and Cockburn 1995; Logue 2007). Traditionally, female song has been correlated with a number of life history traits that are more frequent in tropical regions, including year-round territoriality, lack of migration, monogamy, and sexual monochromatism (Najar and Benedict 2015). Female song in temperate regions is thought to be far less common, but it may be more difficult to detect and document. Many long-held assumptions about female song are currently being reexamined as it is documented in an accumulating number of species.

Female song was once believed to be primarily a non-adaptive hormonal aberration (Langmore 1998), but there is building evidence that it can have an adaptive function in both inter- and intra-sexual communication (Odom et al. 2014). Female song appears to function in mate guarding (Yasukawa and Searcy 1982), mate attraction (Langmore et al. 1996), and in the coordination of reproduction (Ritchison 1983; Sonnenschein and Reyer 1983). In addition, it may function intersexually for territorial defense or as a means of reinforcing male-female pair bonds (Farabaugh 1982; Sonnenschein and Reyer 1983), and it may be more common when competition for resources is high (Arcese et al. 1988). Nonetheless, the mechanisms underlying female song and the reasons selection has favored the apparent loss of female song in many species remain poorly understood.

Female song has been reported in approximately 23% of species within the New World wood-warblers (family Parulidae), which includes both resident and migratory species (Najar and Benedict 2015). Parulid species with reports of female song are about equally represented

among tropical and temperate regions; however, frequent female song, such as duetting, is most common in non-migratory species inhabiting tropical regions (Najar and Benedict 2015). Other than a single mention in the *Birds of North America* without any data to corroborate the statement, female song in the Cerulean Warbler (*Setophaga cerulea*) lacks documentation (Buehler et al. 2013). Here we describe the occurrence of song in 2 mated female Cerulean Warblers, discuss its context, and hypothesize on a potential adaptive function in this species.

3. METHODS

We monitored a breeding population of Cerulean Warblers in southern Indiana, USA, from May-July 2017. Cerulean Warblers at our study sites have been closely observed for 11 breeding seasons (2007-2017) as part of the Hardwood Ecosystem Experiment, a long-term (100 years) multi-taxa, multi-organizational study initiated in 2006 (Swihart et al. 2013). Two female Cerulean Warblers were observed to sing and were recorded by G.J. MacDonald and C.D. Delancey. These individuals occurred in Yellowwood and Morgan-Monroe state forests in Brown and Monroe counties and were separated by greater than 20 km. One female (hereafter, Female 'A') was documented singing from 14-22 June 2017, a period that mostly overlapped with the incubation period of her nest. The other female (hereafter, Female 'B') was documented singing from 20-21 June 2017, which corresponded to the late nestling stage of her nest. Cerulean Warbler nest transition dates in our system are approximate because nests at our field sites are generally high in the canopy (average 18 m) and their contents cannot be observed from the ground (Wagner and Islam 2014). Cerulean Warblers are sexually dichromatic and were visually sexed at the time of each observation. In each instance, we also confirmed sex through the repeated observation of incubation or brooding, which are female-specific behaviors in this

species (Buehler et al. 2013). At both nests, a male plumaged bird also tended the nest, which further confirmed the sex of each female.

The songs of the 2 females were recorded opportunistically using a Marantz PMD-661 MKIII solid-state digital recorder (48 kHz sampling rate, 24-bit) with a Sennheiser ME62/K6 microphone and a Telinga Pro Universal parabola. Additionally, C.D. Delancey obtained video of Female 'A' while she sang using a Nikon D5300 DSLR camera and a Tamron 150-600 mm lens mounted on a Vanguard ABEO 243AV tripod. We also obtained audio recordings from Wildlife Acoustics SM4 automated recording units (hereafter, ARU; 48 kHz sampling rate, 16-bit) that we deployed in mid-canopy forest within 15 horizontal meters and 10 vertical meters of each nest for a different project. ARUs recorded from 1 hour prior to sunrise until 1300 hours for the duration of each nest. The archived audio recordings are available through the Macaulay Library at Cornell University (www.macaulaylibrary.org, catalog numbers: ML Audio 71459011, 71462341, and 71461751) and the video can be found at the following link (https://youtu.be/6HiwANcmWAE). Spectrograms were generated using Raven Pro 1.5 (Bioacoustics Research Program 2014) with the following parameters: a 320-point Hann window with a 4096-point Discreet Fourier Transform (DFT; Female 'A'), a 560-point Hann window with a 1024-point DFT (Female 'B'), and a 460-point Hann window with a 1024-point DFT (the mate of Female 'A').

4. RESULTS

4.1 Form of Female Song

Spectrograms of the songs of both females resembled neither each other, nor a typical male song (Fig. 1). Further, neither song resembled any previously documented Cerulean

Warbler vocalization. In Cerulean Warblers, typical vocalizations include: the male song, which is a series of 1-2 phrases of rapidly uttered repeated syllables followed by a final buzzy trill (Fig. 1C), as well as variations of "*zeet*" and "*chip*" calls, apparently used by both sexes in a variety of contexts (Rogers 2006; Buehler et al. 2013). In comparison, the songs of Female 'A' consisted of a rapid repetition of similarly frequency-modulated notes, and sounded rough and buzzy in quality (Fig. 1A). Her songs were consistently similar in structure, averaging approximately 0.8-1.2 s in duration (song bout) and spanning approximately 4-8 kHz in frequency. Furthermore, her songs had a distinctive pattern of several similar phrases separated by very short inter-phrase pauses. In contrast, the songs of Female 'B' consisted of a series of high frequency, slurred rising and falling notes with a distinct harmonic structure (Fig. 1B). Her songs were approximately 1.5 s in duration and spanned approximately 3-6.5 kHz in frequency. Additionally, the songs of Female 'B' were slightly more variable in both structure and duration than those of Female 'A,' though all songs had the same slurred quality and harmonic overtones.

4.2 Context of Female Song

Female Cerulean Warblers often give a "*zeet*" call from the nest, usually uttered immediately after song from the male (Buehler et al. 2013). Female 'A' mostly replaced these "*zeet*" calls with her songs, which were typically given in extended song bouts from her nest. She normally sang only after the conclusion of a song from her mate, though on occasion she sang spontaneously (i.e., not after her mate's songs). She gave most songs while sitting on her nest, but we also recorded her singing away from her nest while foraging within her mate's territory. Based on 48.6 hours of ARU recordings from 16-22 June 2017, she sang throughout the daily recording period averaging 25.5 songs per hour for a total of 1,238 songs. She abruptly stopped

singing around the estimated hatch day, and she was not observed to sing again despite continued nest observations, as well as spectrographic analysis of the audio recordings from the ARU near her nest.

We were only able to record 9 songs from Female 'B.' All of her songs were given while she foraged near her nest in close proximity to her mate during the late nestling stage. She was never documented to sing earlier in the nesting cycle nor while sitting on her nest, despite 30 min nest observation periods after nest discovery (nest building stage) and spectrographic analysis of ARU audio recordings.

5. DISCUSSION

We report on the first documentation of female song in the Cerulean Warbler. We have chosen to call these vocalizations "songs" based on their duration, complexity, and structure - ("long, complex, vocalizations…produced in long spells with a characteristic diurnal rhythm") - despite their apparent non-use during the mate acquisition period (Catchpole and Slater 2008). The songs produced by the 2 females were distinct from those of their mates and did not resemble normal vocalizations by either sex of this species. They also did not resemble the vocalizations of any other locally breeding bird species, nor any species with which we are familiar. Unlike most other instances of female song in temperate-breeding wood-warblers, we documented singing behavior from both females only during the incubation or nestling stage rather than during the mate acquisition period. Female 'A' sang for at least 9 days, which largely overlapped with incubation, and Female 'B' apparently sang only late in the nestling stage. It is possible that we missed earlier instances of song by either or both females, but we believe this to be unlikely because their nests were monitored for 30 minutes every 1-3 days from discovery (both in the nest building stage) until the completion of the nest. Additionally, before we first

heard song from Female 'B,' we had previously deployed an ARU near her nest for a separate project. Subsequent analysis of recordings spanning 30 May-5 June failed to reveal song previous to 20 June, when we first recorded her songs.

Given the context in which female song was given in the presence of each female's mate, we hypothesize that female song in these instances may have functioned inter-sexually in reinforcing the male-female pair bond during a period of heightened resource competition. Female 'A' often sang in the presence of her mate and her songs were typically given immediately following the completion of his song. Additionally, Female 'A' almost completely replaced the typical female *"zeet"* call, which is given from the nest, with her songs. We failed to detect this same behavior in Female 'B,' but her songs were always given in close proximity to her mate during feeding bouts to their nest. They may have served to reinforce intersexual communication during the nestling stage, a stressful and energetically demanding time for adult birds.

Female song in the family Parulidae is best known in species breeding in tropical or sub-tropical regions and in species that are sedentary or sexually monochromatic (Najar and Benedict 2015). Additionally, it may be more frequent in instances where resource competition is intense (Arcese et al. 1988). Cerulean Warblers, however, do not conform to this pattern: they breed in temperate eastern North America, are completely migratory, and are highly sexually dichromatic. Little is known about inter- and intra-specific competition for resources involving Cerulean Warblers, though as cup-nesters, they may experience less competition than would a cavity-nesting wood-warbler (Lindell 1996). Anecdotally, we noted that the nest location of Female 'A' at our study site was in a "hotspot" of Cerulean Warbler activity; nest densities in this area were high relative to other areas at our study sites, with at least 4 other Cerulean Warbler territories

within 100 m. Contrastingly, Female 'B' nested in a low-density breeding location; the nearest Cerulean Warbler breeding pair was greater than 0.5 km away. Nonetheless, female song in Cerulean Warblers may function inter-sexually in reinforcing the male-female pair bond when resource competition is high, though this remains an important question for future research.

Cerulean Warblers have been studied intensively in many areas of their breeding range, including 11 consecutive years of research by our laboratory at the same study sites in southern Indiana; therefore, it is surprising that this behavior has not been documented previously. There is 1 prior report of female song in this species (without documentation), a bird in Desha County, Arkansas in May 2006 that was noted to be in female plumage (Buehler et al. 2013). It should be noted, however, that some second-year male Cerulean Warblers are extremely dull and female-like in plumage, and thus confusion regarding sex can occur in rare instances (pers. observ.). Breeding density and resource competition may play a role in the occurrence of female song in this species, as well as in other species where females do not normally sing. In order to better understand its function, female song should be looked for in other populations of Cerulean Warblers, particularly those with high breeding densities.

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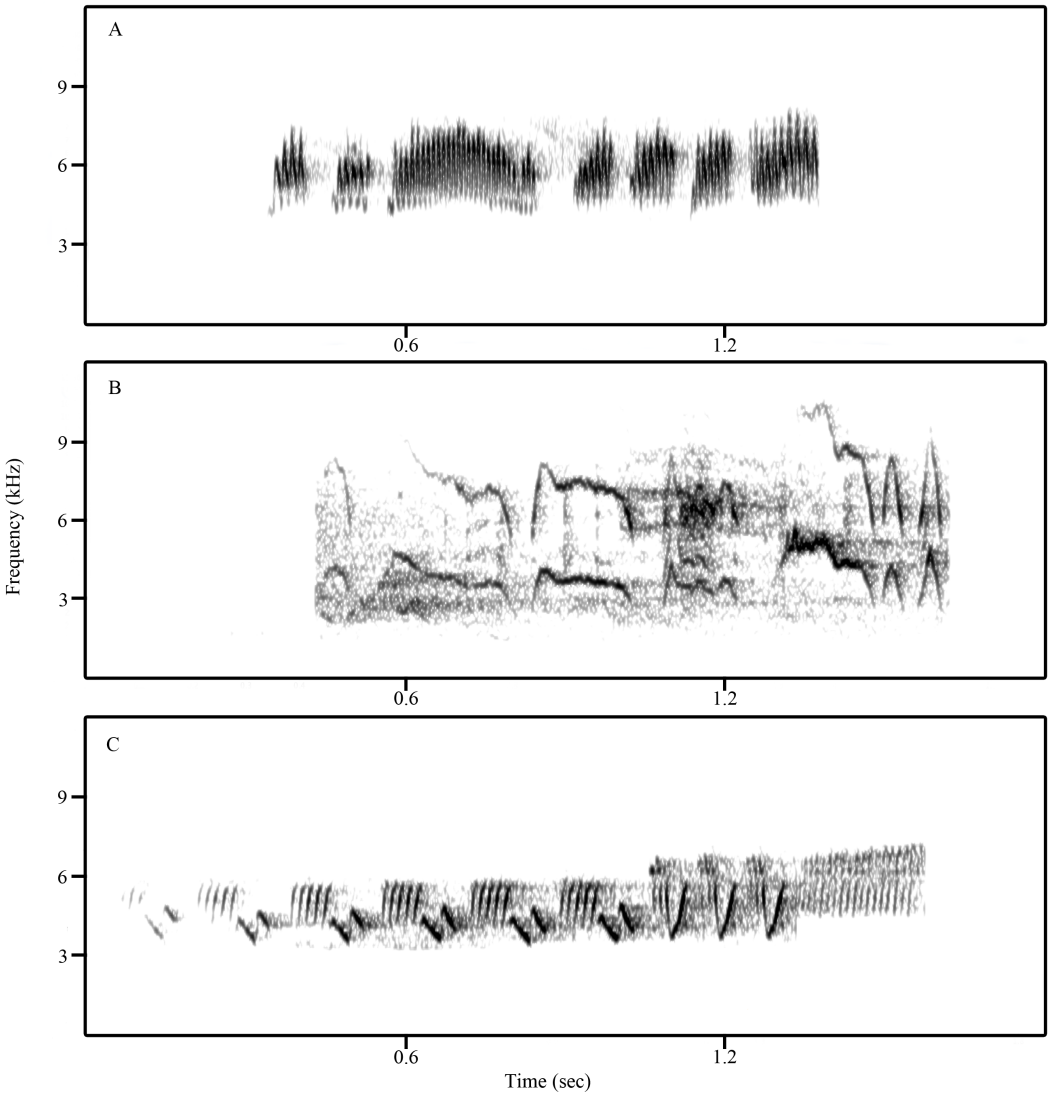
CHAPTER 4 FIGURES

Figure 1. Spectrograms of the songs of male and female Cerulean Warblers. A) Typical song sequence from Female 'A.' B) Song from Female 'B.' C) Typical Cerulean Warbler male song; specifically, the song produced by the mate of Female 'A.' Recordings are available from the Macaulay Library at Cornell University (www.macaulaylibrary.org, catalog numbers: ML Audio 71459011, 71462341, and 71461751).



CHAPTER 5: First confirmed hybrid pairing between a Cerulean Warbler (*Setophaga cerulea*) and a Black-throated Blue Warbler (*Setophaga caerulescens*)

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1. ABSTRACT

Hybridization events have long held the interest of biologists because of implications for species concepts and taxonomy, and rates of hybridization are commonly used as support for accepted taxonomic divisions. Therefore, it is important to document new cases of hybridization among species when they are encountered. In July 2017, we observed a nest where a male Cerulean Warbler (*Setophaga cerulea*) and a female Black-throated Blue Warbler (*Setophaga caerulescens*) were feeding nestlings. The Cerulean Warbler and Black-throated Blue Warbler communicated with each other via song (male) and calls (female), much as a non-hybrid pair of either parent species would, and the pair never engaged in aggressive behaviors toward each other. Previously, the Cerulean Warbler has only been documented to hybridize with the Northern Parula (*Setophaga americana*) and the Black-and-white Warbler (*Mniotilta varia*), and the Black-throated Blue Warbler has been documented hybridizing with the Yellow Warbler (*Setophaga petechia*) and Nashville Warbler (*Leiothlypis ruficapilla*). Interestingly, the Black-throated Blue Warbler is not known to breed in Indiana, but it is a regular migrant in the spring and fall. We describe our observations of the behaviors of the hybrid pair at the nest, provide

photographs and audio recordings for documentation, and provide explanations on the origins of this rare instance of pairing.

Keywords: Black-throated Blue Warbler, Cerulean Warbler, hybrid, Parulidae, *Setophaga caerulescens*, *Setophaga cerulea,* wood-warbler

2. INTRODUCTION

Of the 47 species of wood-warblers that breed in North America, 34 have been reported to hybridize (McCarthy 2006). Globally, approximately 9.2% of bird species have been observed in hybridization events (Grant and Grant 1992), so the incidence rate of hybridization of >50% in North American parulid warblers is quite high. Hybridization occurrences have long been important in the species concept, and hybridization rates provide important evidence in taxonomy. It is a rare occurrence, however, and even rarer to actually observe a hybrid pairing event in nature (Willis et al. 2014).

According to McCarthy (2006), the Cerulean Warbler (*Setophaga cerulea*) has been known to hybridize with the Black-and-white Warbler (*Mniotilta varia*) and the Northern Parula (*Setophaga americana*). The first record of a hybrid with a Cerulean Warbler was collected on 24 April 1954 (Parkes 1978). This hybrid was an adult male Black-and-white Warbler x Cerulean Warbler. Comparisons were made between after-second-year Cerulean Warbler and Black-and-white Warbler. Parkes (1978) noted differences and similarities between colorations and patterns of every body part (crown, face, back, rump, upper tail coverts, tail, throat, breast band, sides, flanks, under tail coverts, and wings). Other measurements taken from the specimen included flattened wing, tail, bill from anterior edge of nostril, tarsus, hallux minus claw, chord of hind claw, hallux, claw/tarsus and weight. This hybrid specimen mostly resembled a Black-and-white Warbler, but the white stripes of the upperparts were replaced by a blue-gray color (Parkes 1978). It also had a white throat and a black band across the upper breast. This specimen proved to be intermediate between Black-and-white Warbler and Cerulean Warbler upon conclusion of the study (Parkes 1978).

There are several previously documented instances of hybridization between Cerulean Warbler and Northern Parula. In one case in East Hampton, Long Island, NY, the possibility of a hybrid pair between the two species proved no solid evidence. A male Northern Parula was documented feeding nestlings, but at one point it was chased away by the female Cerulean Warbler that owned the nest. A male Cerulean Warbler and a female Northern Parula were never seen in the area near this nest (Lindsay and Vezo 1995). A more interesting report from Hyde Park, Dutchess County, NY, reported an unusually plumaged Cerulean Warbler. Before this bird was seen, it was heard vocalizing, and identified as a Northern Parula based on its song. When first seen, it mostly resembled a Cerulean Warbler, but it sang a Northern Parula song (Pooth and Johnson 2004). The authors described the bird's body plumage as that of a Cerulean Warbler (blue above, white below with wing bars, and a thin blue-gray necklace). They noted that the head was more like a Northern Parula, completely blue, along with a distinct broken eye-ring. The bird did not have any streaking on its sides or flanks. In a later observation, the bird sang like a Northern Parula, but occasionally sang songs typical of a Cerulean Warbler (Pooth and Johnson 2004).

On 16 May 2004, another unusually plumaged bird was observed, this time in Toledo, Ohio. It closely resembled a Cerulean Warbler, but it did not have a breast band or distinct side-streaking. It had a distinct broken eye-ring, characteristic of Northern Parula, but there was no yellow on the throat or breast, and it lacked a greenish back (Nirschl 2004). The blue on the forehead, crown, and wings was described as a cerulean blue, rather than the typical blue color of a Northern Parula. When first observed early in the summer, it was singing the primary song of a Northern Parula, but on 30 June 2004, it was observed singing a Cerulean Warbler song. On 1

July 2004, the bird was heard singing both Cerulean Warbler and Northern Parula songs (Nirschl 2004).

Multiple Cerulean Warbler x Northern Parula hybrids have been documented with photographs and audio recordings on eBird (eBird 2012). These individuals were determined to be males based on plumage characteristics and singing behavior, and most individuals showed plumage characteristics that were intermediate between both species. Most individuals had broken eye-rings, faint breast bands, and with upper parts that were cerulean blue in color. The crown coloration of these individuals was often monotone and did not have multiple colors like that of a Cerulean Warbler. However, some individuals varied in plumage coloration and had a hint of yellow or orange color on the breast, which would suggest a Northern Parula trait (O'Brien 2009; Sanderson 2016; Gilliam 2017; Ivanov 2017; Wistrand 2017a).

The Black-throated Blue Warbler (*Setophaga caerulescens*) has been documented to hybridize with Yellow Warbler (*Setophaga petechia*; Ducharme and Lamontagne 1992), and with Nashville Warbler (*Leiothlypis ruficapilla*; Dunn and Garrett 1997; Williams 1996). Here, we report the first hybrid pairing event between a Cerulean Warbler (male) and a Black-throated Blue Warbler (female). We provide our observations of the pair at the nest and discuss possible explanations for this hybrid pairing.

3. METHODS

These observations were made on private property in Brown County, Indiana (39°13'42.44"N, 86°11'10.16"W) from 7-8 and 12-13 July 2017. Matthew Wistrand first discovered the nest on 7 July (Wistrand 2017b) and then subsequently observed the nest on 8 July before departing the area (Wistrand 2017d). The authors were allowed access to the property from 12-13 July. Once on the property, it did not take long to locate the reported nest

based on detailed directions (Mathew Wistrand, pers. comm.). We documented the hybrid pairing with photographs (Nikon D5500 with a Tamron 150-600 mm lens) and audio recordings (Marantz PMD 661 MKIII solid-state digital recorder and Sennheiser ME62/K6 microphone with a Telinga Universal Pro parabola).

4. RESULTS

A nest was found on 7 July 2017 on private property in Brown County, Indiana by Matthew Wistrand, a visiting birder, who submitted an eBird checklist with a description of his observations (Wistrand 2017b). Most notably, he wrote about how a female Black-throated Blue Warbler was paired with a male Cerulean Warbler attending to a nest. Wistrand noted that the male Cerulean Warbler visited the nest to feed either the female Black-throated Blue Warbler or young nestlings (his views of the nest were poor). Included in that checklist were poor quality photographs showing the female Black-throated Blue Warbler (Wistrand 2017b). The only distinguishing characteristic in the photographs was the white wing patch of the female Black-throated Blue Warbler. Wistrand also obtained audio recordings of the female Black-throated Blue Warbler and uploaded them to xeno-canto (Wistrand 2017b, Wistrand 2017c). Wistrand revisited the nest site on 8 July 2017, and he observed both the female Black-throated Blue Warbler and the male Cerulean Warbler at the same nest. This time he took a poor quality photograph of the male Cerulean Warbler at the nest (Wistrand 2017d).

On 12 July 2017, we obtained permission to visit the nest site to confirm Wistrand's reports. We monitored the nest from 1410 h to 1515 h and counted four nestlings (Fig. 1b); we remained as far as possible from the nest to minimize disturbance to the parents. One of the first birds we observed after finding the nest was the female Black-throated Blue Warbler, and we obtained audio recordings of her "chipping" near the nest that we later uploaded to eBird

(MacDonald 2017). Soon after locating the female Black-throated Blue Warbler, we located a singing male Cerulean Warbler, and watched him visit the nest, where he fed the nestlings (Fig. 1c, d). The female Black-throated Blue Warbler "chipped" actively while she foraged. During our observation, we noted that the female Black-throated Blue Warbler fed nestlings approximately ten times (Fig. 1e, f), while the male Cerulean Warbler fed nestlings about five times. The male would almost always feed the nestlings after singing. Throughout the observation, many photographs were taken of these individuals, mostly at the nest, which will be uploaded to an eBird checklist at a future date (Delancey 2017). The nest was located about 6 m above ground in a sugar maple (*Acer saccharum*) that had a dead, broken top hanging down. The nest was mostly surrounded by dead leaves from within the broken-off top (Fig. 1a). The nest itself was connected to gravepine (*Vitis sp.*) that was growing on the sugar maple. We noticed that this nesting location was atypical of both Cerulean Warbler and Black-throated Blue Warbler nest placements. The nestlings appeared no more than 4 days from fledging, and often begged loudly (captured in audio recordings). The female Black-throated Blue Warbler was observed brooding the nestlings for about two minutes on occasion. The male Cerulean Warbler and female Black-throated Blue Warbler never interacted aggressively; in fact, they were observed multiple times at the nest simultaneously (Fig. 1g). No other species were seen near the nest during our observation.

On 13 July 2017, we returned after receiving permission to capture these birds to band and collect DNA from all of the individuals. With help from the Streby Laboratory at the University of Toledo, we intended to temporarily remove nestlings from the nest to collect DNA, along with target netting both adults. However, upon arrival we were unable to locate the nest in the tree. The nest was found empty, and on the ground. We could not locate any of the nestlings

that may have survived the nest falling from the tree. We believe the nest was predated as the nest branch had broken off sometime since our visit the previous day. We collected the nest, and as we were leaving the site, we heard a male Cerulean Warbler singing nearby, but we were not able to re-locate the female Black-throated Blue Warbler.

5. DISCUSSION

It is not surprising that the Cerulean Warbler would hybridize with aNorthern Parula. In fact, the Cerulean Warbler and two species of parula (Northern Parula and Tropical Parula *Setophaga pitiayumi*) form a sister group (Lovette et al. 2010). At our research sites in southern Indiana, we observe many Northern Parulas in close proximity to Cerulean Warblers that defend territories in the riparian areas (pers. observ.). Based on feeding habits, nesting habits, plumage, body structure and song, Cerulean Warblers and Northern Parulas are very alike (Pooth and Johnson 2004, Nirschl 2004, Buehler et al. 2013, Moldenhauer and Regelski 2012). However, it is surprising that a Cerulean Warbler hybridized with a Black-and-white Warbler. According to Lovette et al. (2010), the Cerulean Warbler and Black-and-white Warbler are not closely related; they are not even in the same genus. The Black-and-white Warbler is the only species within the monotypic genus *Mniotilta,* which is an older lineage of wood-warbler that is highly distinct from the genus *Setophaga* based on phylogenetic results (Lovette et al. 2010). By examining differences in plumage, behavior, and nesting habits, it is easy to note many strong differences between the Cerulean Warbler and Black-and-white Warbler. Nesting habits between the two species are near-complete opposite of one another; the Cerulean Warbler is a canopy nesting species and the Black-and-white Warbler is a ground-nester. We have observed Cerulean Warbler nests and Black-and-white nests within 15 m of each other in our study area.

At one point, the Cerulean Warbler and the Black-throated Blue Warbler were thought to be "sister species" (Mayr and Short 1970), though they are not currently recognized as such; however, they are both in the genus *Setophaga*. The Cerulean Warbler and the Black-throated Blue Warbler are more closely related to each other however, than either is to the Black-and-white Warbler (Lovette et al. 2010). Holmes et al. (2005) suggested that Mayr and Short (1970) constructed their phylogeny of relatedness based on the blue plumage that both species possess, whereas Lovette et al. (2010) used genetics to construct their phylogeny. The Black-throated Blue Warbler does not have any sister species or close relative (Lovette et al. 2010, Homes et al. 2005).

Hybridization between species may occur in multiple situations. One example is mate rarity (Willis et al. 2014). Since 2007, we have observed a declining population of Cerulean Warblers at our study area (our closest study site is approx. 13.9 km away from the hybrid pair nest). The sex ratio of Cerulean Warblers appears to be skewed in favor of males, as not every territory is occupied by a female (pers. observ.). After an unsuccessful attempt at attracting a mate, a male Cerulean Warbler may pair up with a different species, such as a Black-throated Blue Warbler. At the hybrid pair nest site, we did not observe or hear any other Cerulean Warblers. According to Willis et al. (2014), closely related species are more likely to hybridize with each other than species that are not as closely related. For example, the Northern Parula and the Cerulean Warbler are closely related (Lovette et al. 2010), but it does not quite address hybridization between Cerulean Warbler and Black-throated Blue Warbler. What is even more remarkable is that the Black-throated Blue Warbler is not known to breed in the state.

The hybrid pair's nest was "intermediate" in nest height at about 6 m from the ground. At our research sites, Cerulean Warblers nest at an average height of 18 m (Wagner and Islam

2014), but we have documented nest heights ranging from 9.0-29.5 m (unpubl. data). Preferred nest tree species for Cerulean Warblers include white oak (*Quercus alba*), chestnut oak (*Quercus montana*), pignut hickory (*Carya glabra*), black walnut (*Juglans nigra*) and sugar maple (Buehler et al. 2013, Wagner and Islam 2014, unpubl. data). Cerulean Warblers typically place their nests on horizontal branches, and conceal the nest with leaves from above. Nests are mainly constructed of grapevine and spider web (Buehler et al. 2013). In central New Hampshire, the Black-throated Blue Warbler nests in hobblebush (*Viburnum sp.*; 51%), but also uses American beech (*Fagus grandifolia*) and sugar maple saplings (21% and 9%, respectively; Holmes et al. 2005). Their nests are generally low to the ground averaging 0.44 m. Two nests have been observed above 10 m in height; one at 11 m in a yellow birch (*Betula alleghaniensis*), and another at 17 m in an Eastern hemlock (*Tsuga canadensis*; Holmes et al. 2005). Nests are usually constructed of thin strips of bark, often obtained from birch trees or rotten wood, and then stuck together using spider webbing and saliva (Holmes et al. 2005). In the southern portion of their range, Black-throated Blue Warblers have been documented to use rhododendron (*Rhododendron sp.*) or grapevine in their nests (Holmes et al. 2005, Bent 1953). Sometimes Black-throated Blue Warblers will place nests in a cluster of dead leaves or amongst dry foliage of a large branch that has recently fallen (Holmes et al. 2005, Nice 1930, Walkinshaw and Dyer 1953).

The nesting location of this hybrid pairing most closely resembles that of a Black-throated Blue Warbler. It was located in, and around, mostly dead leaves and was not on a horizontal branch. Both warbler species have been found to favor sugar maple as a nest tree to some extent. The nest material appeared to consist almost exclusively of grapevine and spider webbing, which closely resembles nesting materials used by female Cerulean Warblers. Yellow

birch does not occur in Brown County, Indiana; this species occurs mainly in the northernmost counties in Indiana. River birch (*Betula nigra*) does occur in Brown County, but this species requires wet soils. We did not see any birch trees at this site, nor does it occur in our study area. White birch (*Betula papyrifera*) is also found in Indiana, but it is restricted to the northwestern parts of the state (Jackson 2004). Nest height would be an outlier for both the Cerulean Warbler and Black-throated Blue Warbler, so we classified it as intermediate.

The Black-throated Blue Warbler is an eastern species. It breeds in southeastern Canada, the northeastern United States, and along the Appalachian Mountains. It is not known to breed in Indiana, but migrates through the state during spring and fall. Aside from this report, there are four other eBird sightings of Black-throated Blue Warblers in Indiana during the breeding season. Three of those sightings were from northern Indiana and were minimally documented (eBird 2012). One eBird sighting, in Brown County Indiana, reported an adult male Black-throated Blue Warbler that was caught in a mist-net on 12 July 2017 (Britton 2017). This bird was banded, and it was beginning its pre-basic molt (Patrick Ruhl, pers. comm.), which is unusual because Black-throated Blue Warblers are known to initiate their pre-basic molt while still on the breeding grounds. Black-throated Blue Warblers migrate through Indiana, but the nearest they breed (to the north) is northern Michigan. Their breeding distribution extends from northern Georgia to the Appalachian Mountains into New Brunswick and Nova Scotia, Canada (Holmes et al. 2005). There are 14 records of Black-throated Blue Warblers in Indiana during the summer months that are not on eBird (K. Brock, pers. comm.). Only three of these reports include more than one individual bird reported. However, there are no confirmed breeding attempts of this species in the state of Indiana, until this report. We believe that past reports of Black-throated Blue Warblers in Indiana in the summer were mostly of vagrant individuals or

early fall migrants. Although Indiana's land-use is largely agricultural, some forested areas within the state may provide breeding habitat for Black-throated Blue Warblers. It is possible that some other species may breed uncommonly within the state, but have yet to be discovered. Recently, the first successful breeding of the Chestnut-sided Warbler (*Setophaga pensylvanica*) in 20 years was documented in southern Indiana (Ruhl et al. 2015). On 21 July 2017, we captured a Blue-headed Vireo (*Vireo solitarius*) in a mist-net and discovered that it was a singing male on territory. Its partial brood-patch suggested that it was in breeding condition. Yet, Blue-headed Vireos are not known to breed in southern Indiana (Morton and James 2014). So although some species may not be known to breed in Indiana, or southern Indiana, as long as the habitat is suitable, it is possible that there are some uncommon breeders that are yet to be discovered.

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CHAPTER 5 FIGURES

Figure 1. a) View of the hybrid nest in mixed live and dead foliage. b) Close-up of four nestlings. c) Male Cerulean Warbler with a beak full of green caterpillars. d) Male Cerulean Warbler feeding nestlings. e) Female Black-throated Blue Warbler at the nest. f) Female Black-throated Blue Warbler feeding nestlings. g) Male Cerulean Warbler brings food to nestlings after female Black-throated Blue Warbler has finished feeding young. Photo credits: Clayton Delancey.